INFLUENCE OF TRAP CROPPING AND PREDATOR AVOIDANCE BEHAVIOR ON HABITAT CHOICE PATTERN BY ADULT WHITEFLY (HEMIPTERA: ALEYRODIDAE)

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Doo Hyung Lee, Ph. D.

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This study examined how adult whiteflies modulate their habitat choice patterns under trap cropping (i.e., bottom-up effect) and predation risk (i.e., top-down effect), and how this can influence pest management efficacy. First, eggplant was evaluated as a trap crop for two whitefly species, *Bemisia argentifolii* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), on poinsettia. The results indicate that trap cropping has limited potential for either whitefly species for two different reasons. For *B. argentifolii*, the trap cropping was not effective as this species did not respond to the trap crop if the adults had first settled on the cash crop. For *T. vaporariorum*, high mortality of adult whiteflies on the cash crop significantly diluted the effectiveness of trap cropping even when a large number of adults were attracted to the trap crop. For this reason, there was only a marginal management benefit from the trap cropping for *T. vaporariorum*. Collectively, the trap cropping study demonstrates that the effectiveness of trap cropping is governed by the interplay between pest attraction to a trap crop and pest mortality on a cash crop. Experiments were conducted to determine whether adult *B. argentifolii* avoided predators of their offspring and how this behavior might influence trap cropping efficacy. The presence of predators, *Delphastus catalinae* (Coleoptera: Coccinellidae), on a poinsettia cash crop induced more adult whiteflies to leave the cash crop and move onto a cucumber trap crop,

compared to whiteflies on the cash crop with no predators. This push effect increased the efficacy of the trap crop. Another set of experiments revealed that adult *B. argentifolii* modulated their avoidance behaviors to the predators across different spatial scales. The adult whiteflies delayed their settling on leaf-discs when they are confined with predators compared to when there were no predators. The adults showed a significant avoidance behavior at a within-plant scale by moving into the upper parts of the plants from the lower parts harboring predators. However, the presence of predators did not induce more whiteflies to disperse into adjacent plants at an among-plant scale. This study indicates that adult whiteflies can significantly change their habitat choice patterns under trap cropping and predation risk, and thus this behavioral change needs to be considered in the context of pest management.

BIOGRAPHICAL SKETCH

1. Doo-Hyung was born in 1978 in Seoul, South Korea.
2. He was a tall and shy boy, and grown in the middle of competitive Korean education systems.
3. He liked to play basketball during his middle and high schools, and admired MJ 23 and Chicago Bulls.
4. He was able to get admission from a prestigious university, Korea University.
5. But, he had to choose an agricultural major, Agricultural Biology, as his test score was not enough for medical school.
6. He had been grown as a city boy, so was not immediately fascinated by classes.
7. He enjoyed playing billiard with his friends rather than taking classes seriously.
8. He joined Korean Army after finishing two years at college and served as an honor guard for two years.
9. He was better disciple after the military service and started taking his future seriously.
10. He was lucky to take some pest management classes by Dr. Kijong Cho, who strongly motivated Doo-Hyung to proceed with graduate school to study applied entomology.
11. He was lucky to attend a keynote lecture by Dr. Jan Nyrop in Korea and was inspired again to stretch his education into a graduate program.
12. He did his masters degree with Dr. Cho, and during that time he was able to spend a considerable time to interact with growers and listen to their problem and need.

13. He was able to get admission from Department of Entomology, Cornell University, for his dissertation research with Jan.
14. He was lucky to get married with Hye In and moved together to Ithaca, New York.
15. He started playing tennis and getting excited more and more with the new sport.
16. He did enjoy his Ph.D. programs and dissertation research with whiteflies.
17. God gave him and his wife a beautiful gift, Olivia Choyoon Lee.
18. He has successfully completed his dissertation research.
19. Doo-Hyung is ready to move onto his postdoctoral position in West Virginia, and will continue to work and think hard to function as a PI in the near future.
20. He also plans to play tennis with Olivia in the near future.

사랑하는 나의 가족에게 이 논문을 바칩니다.

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CHAPTER 1

STUDY SYSTEMS AND RESEARCH OBJECTIVES

Pest management practice has shifted from a reliance on chemical pesticide applications to more integrated and environmentally-safe practices. The new management programs include several cultural and biological management tactics. Trap cropping is one of the cultural practices that use behavioral manipulation of target pests to avert their feeding/oviposition pressure from a cash crop to other economically disposable plant materials (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Another approach, biological control, uses third trophic consumers to suppress herbivorous pests and has produced tangible outcomes including commercialized natural enemies for several arthropod pests (Heinz et al., 2004).

Trap cropping and biological control have promising potential as sustainable approaches to manage economically important pests. However, unlike the immediate efficacy of synthetic pesticide applications, the practices have produced mixed outcomes. This is a barrier for growers to widely adopt these practices and is a challenging motivation for researchers to discover the underlying mechanisms of the interactions between target pests versus plants and/or natural enemies.

In the study reported here, my research focused on better understanding of tri-trophic interactions of whiteflies (Hemiptera: Aleyrodidae) and applying the knowledge in the context of pest management. More specifically, the study explored resource use patterns of whiteflies under trap cropping and biological control to

deepen our understanding of the behavior and ecology of the target pests under these management practices. Whiteflies were studied as a target pest because of 1) their economic importance in agriculture as a worldwide crop pest (Oliveira et al., 2001) and 2) an emergent need for alternatives or supplements to synthetic pesticides (Palumbo et al., 2001). In particular, it is noteworthy that a considerable research effort has been made to develop and enhance sustainable management practices for this destructive pest but the practices have produced highly variable outcomes (Faria & Wraight, 2001; Gerling et al., 2001; Hilje et al., 2001; Naranjo, 2001).

Therefore, it is clear that more studies are needed to better understand how whiteflies respond to diverse stimuli that derive from either bottom-up or top-down factors and assess the impact of these responses at the population level. In this study, I addressed two broad questions concerning the habitat use patterns of whiteflies. First, I examined how adult whiteflies respond to a trap crop and how this can influence the efficacy of trap cropping. Second, I examined how adult whiteflies respond to their natural enemies and how this can influence the management efficacy. Under these two main questions, I conducted manipulative experiments and simulation modeling to address the following four objectives:

1. To evaluate eggplant as a trap crop for two co-occurring whitefly species, *Trialeurodes vaporariorum* and *Bemisia argentifolii*, on greenhouse poinsettia and to understand the mechanisms governing the efficacy of trap cropping (Chapter 2).

2. To determine whether host experience by *T. vaporariorum* on a poinsettia cash crop can alter the effectiveness of trap cropping (Chapter 3).

3. To determine whether *B. argentifolii* avoid natural enemies on a poinsettia cash crop and whether this can be used to increase the effectiveness of trap cropping (Chapter 4).

4. To examine how *B. argentifolii* alter their habitat use patterns in response to predators, *Delphastus catalinae*, across different spatial scales (Chapter 5).

REFERENCES

Faria M & Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. Crop Protection 20: 767–778.

Gerling D, Alomar O & Arnó J (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Protection 20: 779–799.

Heinz KM, Van Driesche RG & Parrella MP (2004) Biocontrol in protected culture. Ball Publishing, Batavia, IL, USA.

Hilje L, Costa HS & Stansly PA (2001) Cultural practices for managing *Bemisia tabaci* and associated viral diseases. Crop Protection 20: 801–812.

Hokkanen HM (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119–138.

Naranjo SE (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop Protection 20: 835–852.

Oliveira MRV, Henneberry TJ & Anderson P (2001) History, current status, and collaborative research projects for *Bemisia tabaci*. Crop Protection 20: 709–723.

Palumbo JC, Horowitz AR & Prabhaker N (2001) Insecticidal control and resistance management for *Bemisia tabaci*. Crop Protection 20: 739–765.

Shelton AM & Badenes-Perez FR (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

CHAPTER 2*

ATTRACTION OF *TRIALEURODES VAPORARIORUM* AND *BEMISIA ARGENTIFOLII* TO EGGPLANT, AND ITS POTENTIAL AS A TRAP CROP FOR WHITEFLY MANAGEMENT ON GREENHOUSE POINSETTIA

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Abstract

Trap cropping, though promising, has had little evaluation in greenhouses. This study evaluated eggplant, *Solanum melongena* L. (Solanaceae), as a trap crop for two whitefly species, *Trialeurodes vaporariorum* (Westwood) and *Bemisia argentifolii* Bellows & Perring (both Hemiptera: Aleyrodidae), on greenhouse poinsettia, *Euphorbia pulcherrima* Willd. ex Koltz (Euphorbiaceae). Because the two whitefly species co-occur in greenhouses, a common trap crop for both whiteflies is desirable. When adults were provided a choice between eggplant and poinsettia in a cage, 60% of *B. argentifolii* and 98% of *T. vaporariorum* were observed on eggplant after 3 days. However, when adults were given eggplant after first settling on poinsettia, only 38% of *B. argentifolii* were later found on eggplant, whereas 95% of *T. vaporariorum* moved to eggplant. In a greenhouse experiment, eggplant did not affect either the spatial distribution or density of adult *B. argentifolii* on poinsettias. In contrast, eggplant changed the spatial distribution of *T. vaporariorum* within 3 days by attracting and retaining the adults. However, the attractiveness of eggplant did not result in a reduced number of *T. vaporariorum* on poinsettias compared with

poinsettias in monoculture. Adult *T. vaporariorum* mortality was high on poinsettias and this likely caused the adult density on poinsettias in monoculture to decrease as fast as that under trap cropping. A simulation model was developed to examine how adult whitefly mortality on poinsettia influences trap cropping effectiveness. When the whitefly mortality was high, simulated densities were similar to the experimental data. This reveals an unexpected factor, pest mortality on the main crop, that can influence the relative effectiveness of trap cropping. Our results indicate that eggplant will not work unilaterally as a trap crop for *B. argentifolii*. For *T. vaporariorum*, attraction to eggplant might be useful as a trap crop system when whitefly mortality on the main crop is not high.

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (= *B. tabaci* biotype B) (both Hemiptera: Aleyrodidae), are the most serious arthropod pests of poinsettia, *Euphorbia pulcherrima* Willd. ex Koltz (Euphorbiaceae). Traditionally, whitefly management on poinsettia has relied upon applications of insecticides which, in turn, have usually resulted in whiteflies developing resistance to these compounds. Resistance to insecticides and other negative impacts of pesticide use on human health and the environment have prompted continuous efforts to enhance biological and cultural control of whiteflies (Faria & Wraight, 2001; Gerling et al., 2001; Hilje et al., 2001; Naranjo, 2001).

Trap cropping is a biologically-based alternative or supplement to chemical

pesticides in which a preferred host plant is used to attract insect pests away from a less-preferred main crop (Vandermeer, 1989; Hokkanen, 1991; Shelton & Badenes-Perez, 2006). This non-toxic management tactic can offer significant economic and environmental benefits and can be integrated with other management tactics. However, it is uncertain whether trap cropping is a viable management tool for whiteflies. For *Bemisia* spp., some authors have concluded that there is no evidence that the presence of a preferred trap crop consistently reduces *Bemisia* spp. density on a main crop (McAuslane et al., 1995; Perring et al., 1995; Smith & McSorley, 2000; Smith et al., 2000). However, others have shown that trap cropping results in reduced levels of *Bemisia* whiteflies or *Bemisia*-vectored viruses (Al-Musa, 1982; Ellsworth et al., 1992; Schuster, 2004). Castle (2006) also demonstrated that the use of cantaloupe as a trap crop reduced *B. tabaci* densities on cotton relative to unprotected fields, but concluded that trap cropping was not able to prevent whiteflies from exceeding economic thresholds on the cotton.

For *T. vaporariorum*, there have been few manipulative experiments to assess the potential of trap cropping. Recently, Murphy (2007) reported on the use of eggplant as a trap crop in combination with augmentative releases of whitefly parasitoids (a total release rate of 15–30 m^{-2} over 12 weeks). The author indicated that it was not uncommon to see hundreds of adult *T. vaporariorum* on the eggplants, surrounded by poinsettias with virtually no whiteflies. However, the relative roles of the trap crop and parasitoids were not differentiated in the study.

Most previous studies have been conducted in open field settings; relatively little is known about trap cropping in greenhouse production. Given that whiteflies are

considered the most serious insect pest of greenhouse poinsettias (Stevens et al., 2000), the development of trap crop systems for use in greenhouses warrants more attention. Trap cropping for whitefly management may be more effective in greenhouse poinsettia production for the following reasons. First, air-flow in a greenhouse is generally mild and constant. Although whiteflies can actively control their flight using visual flow fields under mild wind speeds, the insects generate only relatively weak flight forces and inhibit their take-off as wind speed increases (Byrne et al., 1988; Isaacs et al., 1999). Therefore, the greenhouse environment is more favorable for whiteflies to make directed flights toward a preferred trap crop. Second, poinsettia production typically devotes entire greenhouses to the production of only a few poinsettia cultivars over an isolated growing season (i.e., July–December) resulting in a production system with minimal spatial and temporal heterogeneity of stimuli from the plants (Heinz & Parrella, 1994). For *B. tabaci*, complex sensory stimuli reduce decisiveness or cause distraction in host choice, thereby reducing the fidelity of host choice decisions (Bernays, 1999). If a variety of host plants is present, the resulting complex environment might make it difficult for whiteflies to consistently locate and settle on a preferred host plant (e.g., trap crop). Therefore, the fairly homogeneous environment of poinsettia production might facilitate whitefly movement toward and settlement on a trap crop. Lastly, there are few whitefly-transmitted diseases of poinsettia, so problems associated with disease transmission during the dispersal of adult whiteflies from poinsettia to a trap crop would be minimal.

A trap crop should have inherent characteristics that cause differential

attractiveness to insect pests for feeding and oviposition. There is evidence that eggplant, *Solanum melongena* L. (Solanaceae), may serve as a trap crop for both *T. vaporariorum* and *B. argentifolii*. Because the two whiteflies co-occur in many areas (Greenberg et al., 2002), availability of a common trap crop for both whiteflies will be more appealing to growers. Eggplant has been reported as one of the most suitable host plants for both whitefly species, based on their performance on the plant (e.g., life-table parameters) (Van Lenteren & Noldus, 1990; Tsai & Wang, 1996). In addition, adult *T. vaporariorum* show a strong preference for eggplant over other crops (Van Lenteren & Noldus, 1990); however, the extent to which adult *B. argentifolii* prefers eggplant is unclear.

In this study, we experimentally assessed the potential of eggplant as a trap crop for management of the two whiteflies, *T. vaporariorum* and *B. argentifolii*, on greenhouse poinsettia. First, we determined in a cage experiment whether whiteflies prefer eggplant over poinsettia when given a choice between the two plants. Second, we tested whether whiteflies show an equivalent preference for eggplant after they had first experienced poinsettia by measuring the extent to which eggplant could draw adult whiteflies away from poinsettia. Third, we determined in a greenhouse experiment whether the presence of eggplant can consistently reduce whitefly density on poinsettia by attracting and retaining adults. Finally, we conducted a simulation study to better understand the results obtained from the greenhouse experiment.

Materials and methods

Insects

A *T. vaporariorum* colony was started from individuals originally reared on Kentucky wonder bean, *Phaseolus vulgaris* L., since 1971. A *B. argentifolii* (= *B. tabaci* biotype B) colony originated from individuals reared on poinsettia (cv. 'Freedom Red') since 1989. The colonies were maintained separately on kidney bean, *P. vulgaris*, in walk-in growth chambers at 20–27 °C and L14:D10 photoperiod for at least 6 months prior to the experiments. It has been reported that *B. tabaci* shows a significant increase in fecundity three generations after transfer to a new host plant (Van Lenteren & Noldus, 1990). Thus, 6 months is an adequate time for *B. argentifolii* to adapt to bean before use in our experiments.

Unconditional choice test

This experiment was conducted to measure the preference by adult whiteflies for eggplant and poinsettia, and was unconditional because the whiteflies had no prior experience with either host plant. Experimental arenas were created in BugDorm-3120 cages (0.6 × 0.6 × 0.6 m; MegaView Science Education Services, Taichung, Taiwan). One eggplant (cv. 'Baby Bell'; 9 weeks old, 33 cm high) and one poinsettia (cv. 'Freedom Red'; 11 weeks old, 31 cm high) were placed 20 cm apart in a cage. The position of the plant was randomly assigned to the left or right side of the cage. One-hundred adult whiteflies (mixed age and sex) were collected in a 25-ml glass vial capped with a plastic lid and allowed to acclimate in the vial for at least 30 min. The vial was placed on the bottom of each cage between the two plants and the whiteflies were released by removing the vial's lid. We recorded the number of adult whiteflies on the underside of the leaves of each plant by carefully lifting up and tilting the plant

and counting the insects. Observations were made at 1, 6, 24, 48, and 72 h after the release. The test was replicated 10 times for each whitefly species. The experiment was carried out under 23 ± 1 °C and a L14:D10 photoperiod in a research greenhouse (8 × 6.5 m).

The data were analyzed using repeated measures ANOVA because the number of whiteflies was recorded repeatedly over time on the same plant. The response variable was expressed as the number of whiteflies on the eggplant minus the number on the poinsettia. Thus, the response variable represents the preference of whiteflies for either plant with a positive value indicating a preference for eggplant. We used a linear mixed model to estimate both fixed and random effects on the response variable. In the model, the fixed factors were time, whitefly species, and their interaction; the random factor was cage (i.e., experimental unit) from which the repeated measures were taken over time. For the statistical analysis, we used the Proc MIXED procedure in SAS (SAS Institute, Cary, NC, USA). The variation in repeated measurements was modeled using a spatial exponential covariance structure and was selected based on model fit statistics.

Conditional choice test

This experiment was conducted to determine the extent to which eggplant could draw adult whiteflies away from a poinsettia plant when the poinsettia was infested with adult whiteflies. Because whiteflies were initially on poinsettia, we considered this a conditional choice test. Eggplants (cv. ‘Baby Bell’) were 11 weeks old and 33 cm high; poinsettias (cv. ‘Freedom Red’) were 15 weeks old and 32 cm high. To infest

poinsettia with adult whiteflies, 14 poinsettias were placed in each of two screened cages (2 × 1 × 0.7 m) and 1 400 adult *T. vaporariorum* or *B. argentifolii* (mixed age and sex) were introduced into each of the cages. After 24 h, whitefly-infested poinsettias were removed from the cages and the adult whiteflies on each plant were counted. The whitefly-infested poinsettias were then carefully placed in BugDorm-3120 cages containing one uninfested eggplant (P+E combination) or one uninfested poinsettia (P+P combination) inside. The P+P combination was used to estimate a base-level between-plant movement of adult whiteflies. The number of adult whiteflies on each plant was recorded after 1, 6, 24, 48, and 72 h as described above. The test was repeated seven times (two whitefly species × two plant combinations × seven replicates = 28 cages). The experiment was carried out under conditions of 23 ± 1 °C and L14:D10 photoperiod in a research greenhouse (8 × 6.5 m).

The data were analyzed using repeated measures ANOVA as described above; however, the response variable was expressed as the proportion of whiteflies on the uninfested plant (eggplant for P+E; poinsettia for P+P). The proportions were arcsine-transformed to normalize the data and equalize variances. In the model, the fixed factors were time, whitefly species, plant combination, and their interactions; the random factor was cage (i.e., experimental unit) from which the repeated measures were taken over time. The variation in repeated measurements was modeled using a spatial exponential covariance structure which was based on model fit statistics. To estimate the effects of whitefly species and plant combinations, we made multiple mean comparisons among the combinations of the two factors and significance values were adjusted for the multiple comparisons using the SIMULATE option in SAS.

Trap crop trial

This experiment was conducted to determine whether the presence of eggplant could consistently reduce the number of adult whiteflies and their oviposition on a poinsettia crop. We compared the numbers of adult whiteflies and their progeny on poinsettias in a 'trap plant treatment' (48 poinsettias + two eggplants) to the numbers in a 'poinsettia alone treatment' (50 poinsettias). The poinsettia alone treatment was used to estimate intrinsic decrease in whitefly density on poinsettias during the study (10 days); such losses may result from mortality, off-plant dispersal, or other unknown reasons. If eggplant attracts and holds adult whiteflies in addition to the intrinsic decrease, then the whitefly density on poinsettias should decrease faster in the trap plant treatment than in the poinsettia alone treatment.

The experiment was carried out in a research greenhouse with average temperature of 22–24 °C and L14:D10 photoperiod. A greenhouse bay (8 × 6.5 m) was partitioned in half to make two experimental plots by hanging a curtain of Agribon+ AG-19 screen (Green Mountain Transplants, Arundel, ME, USA) (3.5 m high) down the middle of the bay. A greenhouse bay was randomly assigned to a whitefly species. For the poinsettia alone treatment, 50 poinsettias (cv. 'Freedom Red'; 14 or 16 weeks old, 33 or 55 cm high) were placed within a 3 × 6 m rectangular area; for the trap plant treatment, two poinsettias were replaced with two eggplants (cv. 'Baby Bell'; 8 or 13 weeks old, 40 or 51 cm high) (Figure 2.1). Three-hundred adult whiteflies (mixed age and sex) were collected in a 25-ml glass vial, and five vials were placed in the middle of the plot and then opened, resulting in a total of 1 500 whitefly adults being released

(Figure 2.1). After 0.25, 1, 3, 7, and 10 days, the number of adult whiteflies on each plant was recorded as described above. After 15 days, the number of nymphs (second or third instars) was recorded on the five upper leaves of each plant. The experiment was replicated twice.

The spatial distributions of adult whiteflies were visualized on contour maps to evaluate whether the presence of eggplants altered the patterns over time (SigmaPlot 7.0, SPSS). To draw the contour maps, counts of adult whiteflies per plant were standardized by dividing each observation by the maximum count per plant for each observation time. Following standardization, the two data points (the experiment was replicated twice) for each spatial location were averaged. These average values were used to create contour maps for each observation time.

The objective of this experiment was to test whether the presence of eggplant would result in a reduced number of whiteflies on poinsettias, compared with that on poinsettias in monoculture. To test this, the total number of adult whiteflies on poinsettias was analyzed using a linear regression model with time, treatment (trap plant or poinsettia alone), and their interaction as predictors (JMP 7.0, SAS Institute). The total number of adult whiteflies was log-transformed for the linearity assumption of the model. The regression slopes of the two treatments were compared by evaluating the statistical significance of the interaction term of time and treatment. A significant interaction can be interpreted as a significant difference in the rate at which the adult population decreases over time between the two treatments. We did not statistically compare the data for nymphs on poinsettias between the two treatments because we have only two observations for each treatment.

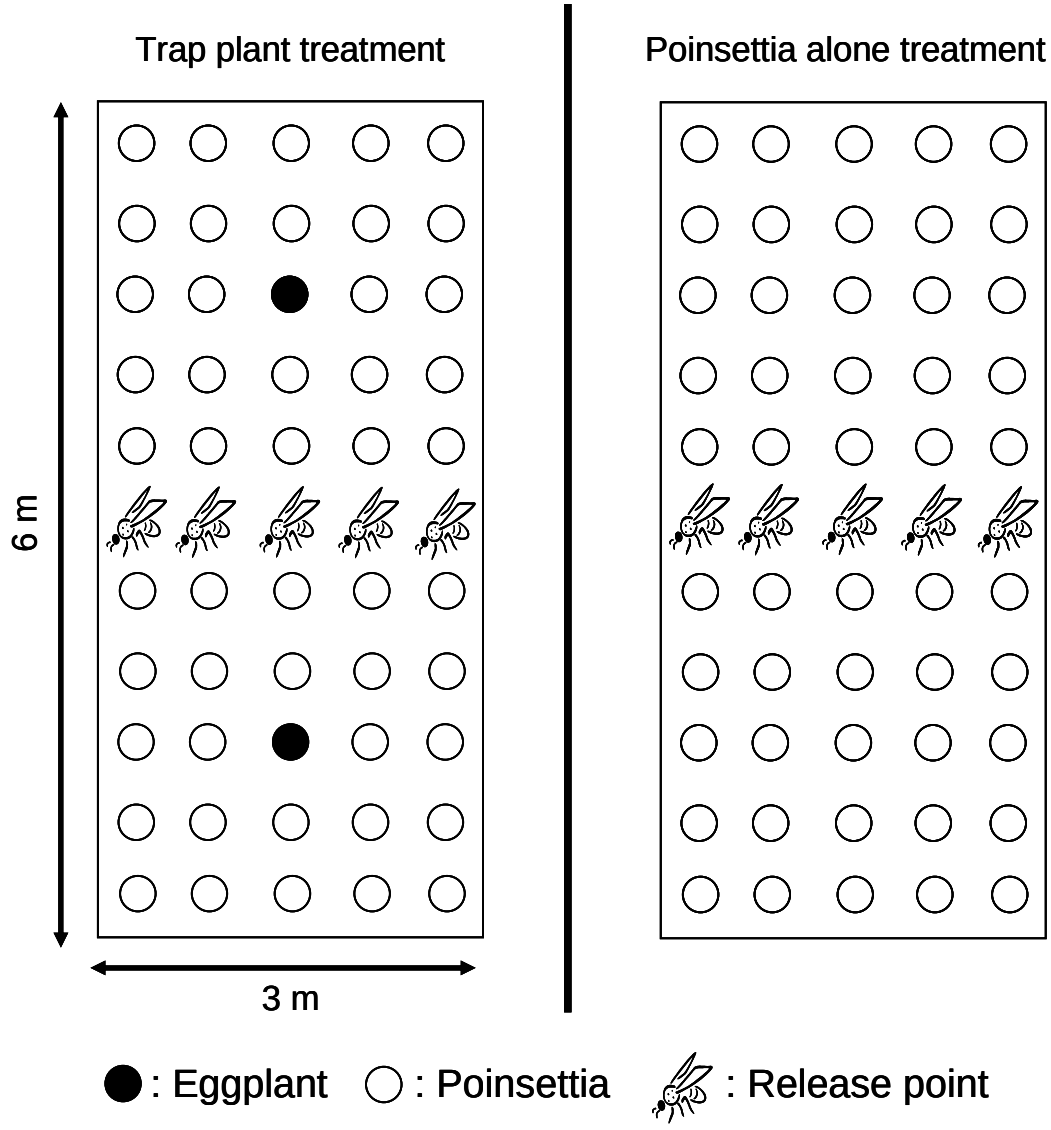

Figure 2.1. Experimental plot layout of the trap crop trial.

Simulation model

We constructed a simulation model of adult whitefly density on poinsettia with and without trap cropping to facilitate understanding of the results from the trap crop trial. The model was constructed as difference equations; however, within each time step of the model we incorporated several processes, each of which was represented by a parameter. A diagrammatic representation of the model is shown in Figure 2.2. Within each time step, a proportion (d) of adult whiteflies on poinsettia become a group of dispersing individuals. We assumed that this proportion is unaffected by the presence of a trap crop. In the absence of a trap crop, a proportion (m) of the dispersing whiteflies die before settling again on a poinsettia plant. Therefore, the number of whiteflies on poinsettia (x) in the absence of a trap crop is determined as

$$x_{t+1} = x_t (1 - d) + x_t d(1 - m), \quad (1)$$

where t is time.

When a trap crop is present, two additional processes are included in the model. First, of those dispersing adults that were to die, a proportion (e) discover an eggplant and hence are saved from death. Our cage experiments indicated that adult *T. vaporariorum* that would have otherwise died, survive on eggplant (see Results). This process can be envisioned as either an attraction to eggplant or, upon discovering an eggplant, a cessation of additional dispersal and hence survival. Thus, when a trap crop is present, the number of whiteflies that die (y) during a time step is given by

$$y_{t+1} = x_t dm(1 - e). \quad (2)$$

Note that this assumes there is no dispersal from eggplant back to poinsettia.

The number of whiteflies on poinsettia is influenced by a second process that

must be modeled when a trap crop is present. Of those whiteflies that disperse and would survive to the next time step by settling on poinsettia, a proportion (e) instead settle on an eggplant and remain there. Thus, the number of whiteflies on poinsettia (x) in the presence of a trap crop is given by

$$x_{t+1} = x_t (1 - d) + x_t d(1 - m) (1 - e). \quad (3)$$

Note that the number of whiteflies on eggplant (z) can be calculated as

$$z_{t+1} = x_t dme + x_t d(1 - m) e = x_t de. \quad (4)$$

Results

Unconditional choice test

Both whitefly species showed a preference for eggplant compared to poinsettia; however, the pattern of preference by the two species was very different (Figure 2.3). The number of adult *T. vaporariorum* on eggplant was significantly greater compared to poinsettia for all observation times ($t = 14.27$, d.f. = 18, $P<0.0001$), and increased over time ($F_{4,45} = 11.83$, $P<0.0001$). Likewise, a greater number of adult *B. argentifolii* were observed on eggplant compared to poinsettia for all observation times ($t = 3.12$, d.f. = 18, $P = 0.0060$); however, this difference was constant over time ($F_{4,45} = 0.25$, $P = 0.91$) (Figure 2.3B). The preference by *T. vaporariorum* for eggplant was much greater than that of *B. argentifolii* ($F_{1,18} = 62.25$, $P<0.0001$).

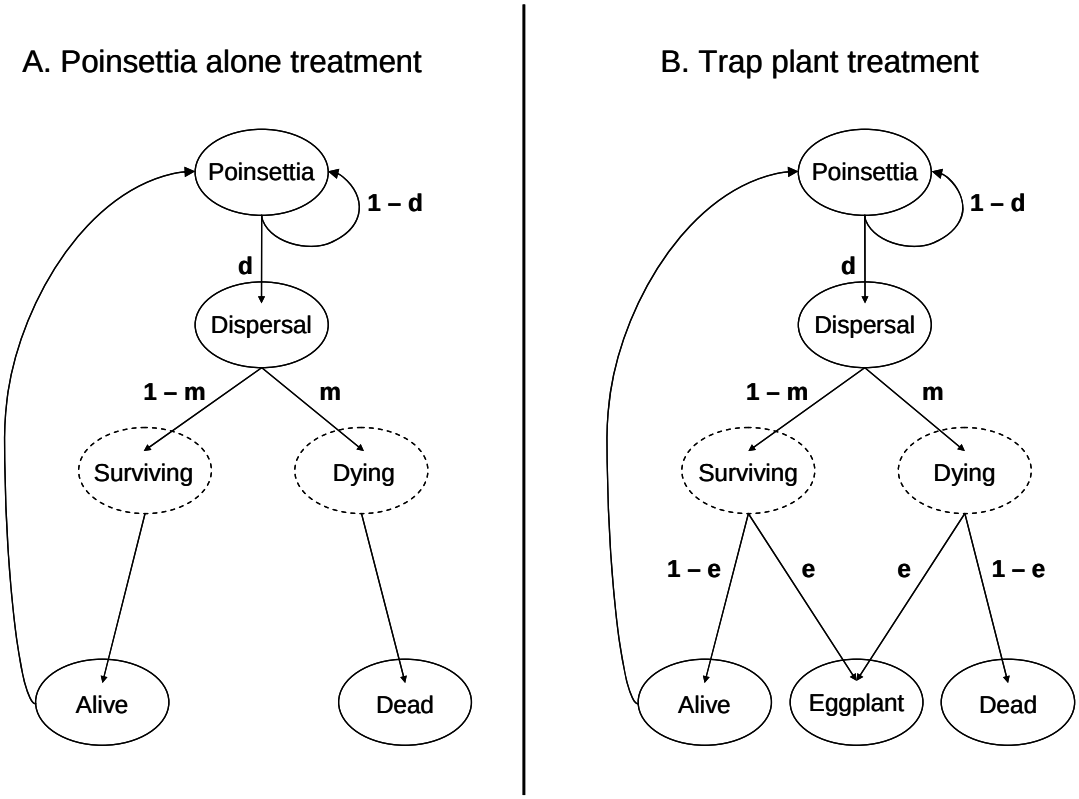

Figure 2.2. Simulation model flow charts. (A) Poinsettia alone treatment. (B) Trap plant treatment.

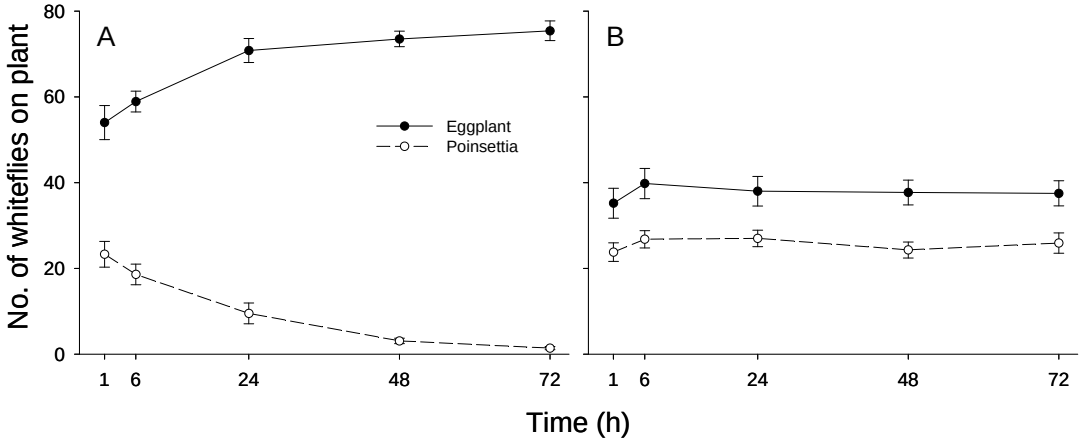

Figure 2.3. Mean number (± SEM) of adult whiteflies observed on eggplant or poinsettia over time in the unconditional choice test. (A) *Trialeurodes vaporariorum* and (B) *Bemisia argentifolii*.

Conditional choice test

More adult whiteflies of both species moved from infested poinsettia to eggplant than from infested poinsettia to uninfested poinsettia. However, *T. vaporariorum* did so to a much greater extent than *B. argentifolii* (Figure 2.4A). In contrast to *B. argentifolii* (see below), *T. vaporariorum* movement from infested poinsettia to uninfested eggplant (P+E) exceeded movement from infested poinsettia to uninfested poinsettia (P+P) during the entire period of the study ($t = 6.32$, d.f. = 24, $P<0.0001$) and the proportion of *T. vaporariorum* on the uninfested eggplant increased to 0.95 after 3 days. This increase resulted from a continuous movement of *T. vaporariorum* from the infested poinsettia to the uninfested eggplant as the total number of the adults observed on the two plants remained constant over time ($F_{4,30} = 0.22$, $P = 0.92$) (Figure 2.4B). In the P+P combination, there was an increase in the proportion of *T. vaporariorum* on the uninfested poinsettia, but the proportion increased to only 0.45 after 3 days (Figure 2.4A). The total number of the adults observed on the two plants decreased over 3 days from 37.86 to 7.00 in the P+P combination ($F_{4,30} = 10.11$, $P<0.0001$) (Figure 2.4B).

For *B. argentifolii*, a greater proportion of the adults was observed on the infested poinsettia than on the uninfested plant in both plant combinations throughout the observation time (Figure 2.4A). The proportion of *B. argentifolii* on the uninfested eggplant (P+E) increased over 3 days to 0.38, and the proportion on the uninfested poinsettia (P+P) increased to 0.19. The proportions were not significantly different when compared across the observation time ($t = 2.16$, d.f. = 24, $P = 0.16$); however, the difference was significant after 3 days ($F_{1,12} = 7.7688$, $P = 0.016$). The total

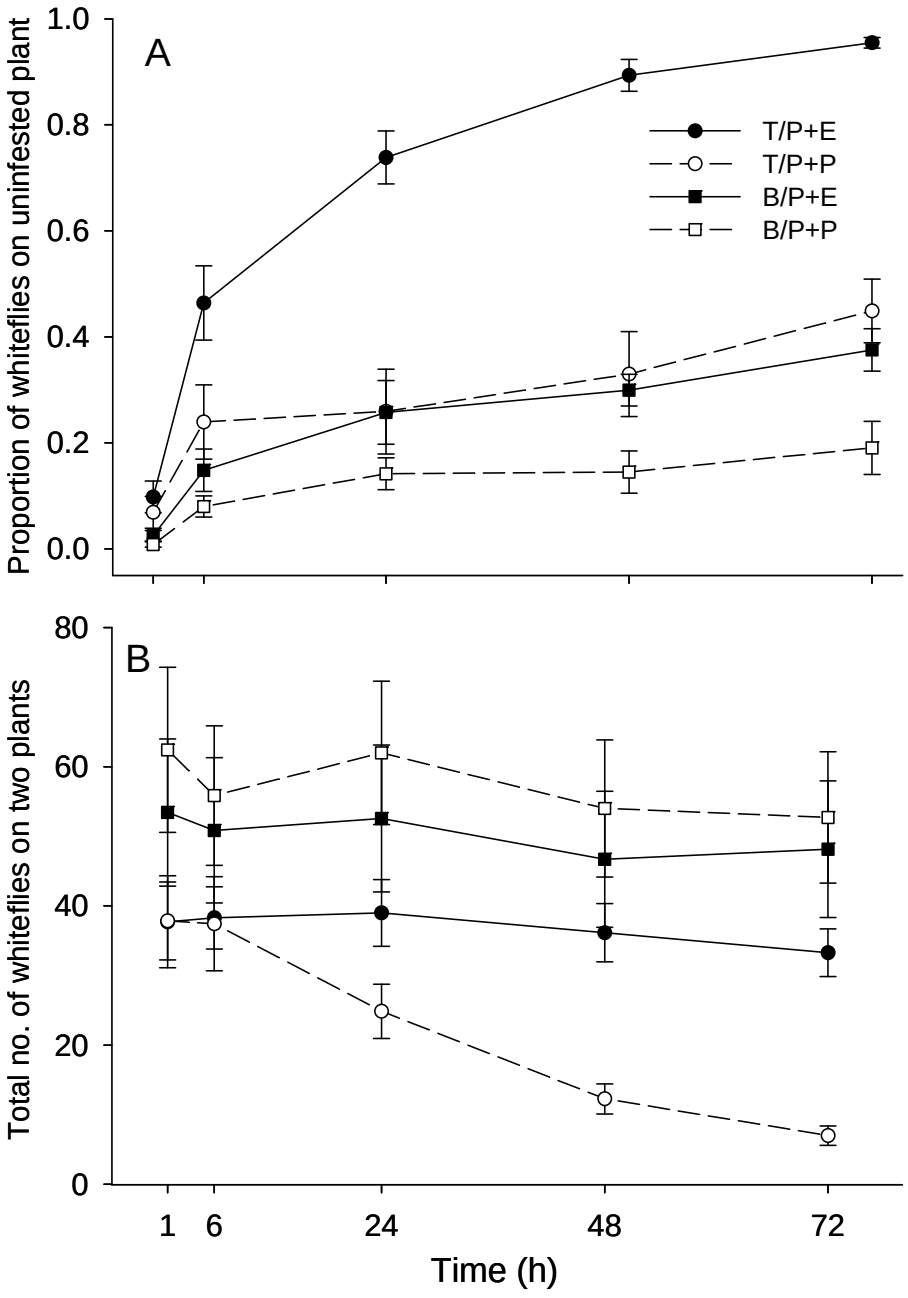

Figure 2.4. (A) Mean proportion (± SEM) of adult whiteflies observed on the uninfested plant over time in the conditional choice test. (B) Mean number (± SEM) of total adult whiteflies observed on both infested and uninfested plants over time in the conditional choice test. T, *Trialeurodes vaporariorum*; B, *Bemisia argentifolii*; P+E, poinsettia + eggplant combination; P+P, poinsettia + poinsettia combination.

number of adults observed on the two plants remained constant over time in both plant combinations (P+E: $F_{4,30} = 0.08$, P = 0.99; P+P: $F_{4,30} = 0.19$, P = 0.94) (Figure 2.4B).

When given the uninfested eggplant (P+E), a greater proportion of adult *T. vaporariorum* moved to the eggplant than of *B. argentifolii* (t = 6.93, d.f. = 24, P<0.0001). However, when given only poinsettias (P+P), there was only a marginally significant difference between the two whitefly species in the proportion of whiteflies on the uninfested poinsettia (t = 2.77, d.f. = 24, P = 0.047) (Figure 2.4A).

Trap crop trial

A clear difference in the spatial distribution of adult *T. vaporariorum* was observed in the trap plant treatment vs. the poinsettia alone treatment (Figure 2.5A,B). A large number of *T. vaporariorum* in the trap plant treatment dispersed from poinsettias to eggplants during the first 3 days, whereas the adults in the poinsettia alone treatment showed a diffused aggregation near the release points.

Although a large number of adult *T. vaporariorum* dispersed from poinsettias to eggplants, there was no evidence that the presence of eggplant resulted in a reduced number of adults on poinsettias compared with the number of adults on poinsettias in monoculture (Figure 2.6A,B). A regression analysis was used to determine whether the presence of eggplant led to changes in the rate at which the number of adults on poinsettias decreased over time. The data were analyzed within trial because there was a significant interaction of time and trial in the poinsettia alone treatment ($F_{1,6} = 15.67$, P = 0.0075) (Figure 2.7). Within trial, there was no significant interaction between time and treatment (first trial: $F_{1,6} = 0.20$, P = 0.67; second trial: $F_{1,6} = 5.72$, P =

0.054), indicating that there was no difference in the rate at which the adult *T. vaporariorum* population decreased over time between the two treatments (Figures 2.6 and 2.7). For nymphs, we observed 1.18× (first trial) and 1.64× (second trial) as many on poinsettias in the trap plant treatment compared to the poinsettia alone treatment (Table 2.1).

For *B. argentifolii*, the presence of eggplant did not alter the spatial distribution of the adults thereby showing that adults had no preference for eggplant (Figure 2.5C,D). Accordingly, there was no difference in the rate at which the *B. argentifolii* population on poinsettias decreased between the two treatments across the two trials ($F_{3,12} = 1.07$, $P = 0.40$) (Figure 2.6C,D). For nymphs, we observed 1.26× (first trial) and 0.97× (second trial) as many on poinsettias in the trap plant treatment compared to the poinsettia alone treatment (Table 2.1).

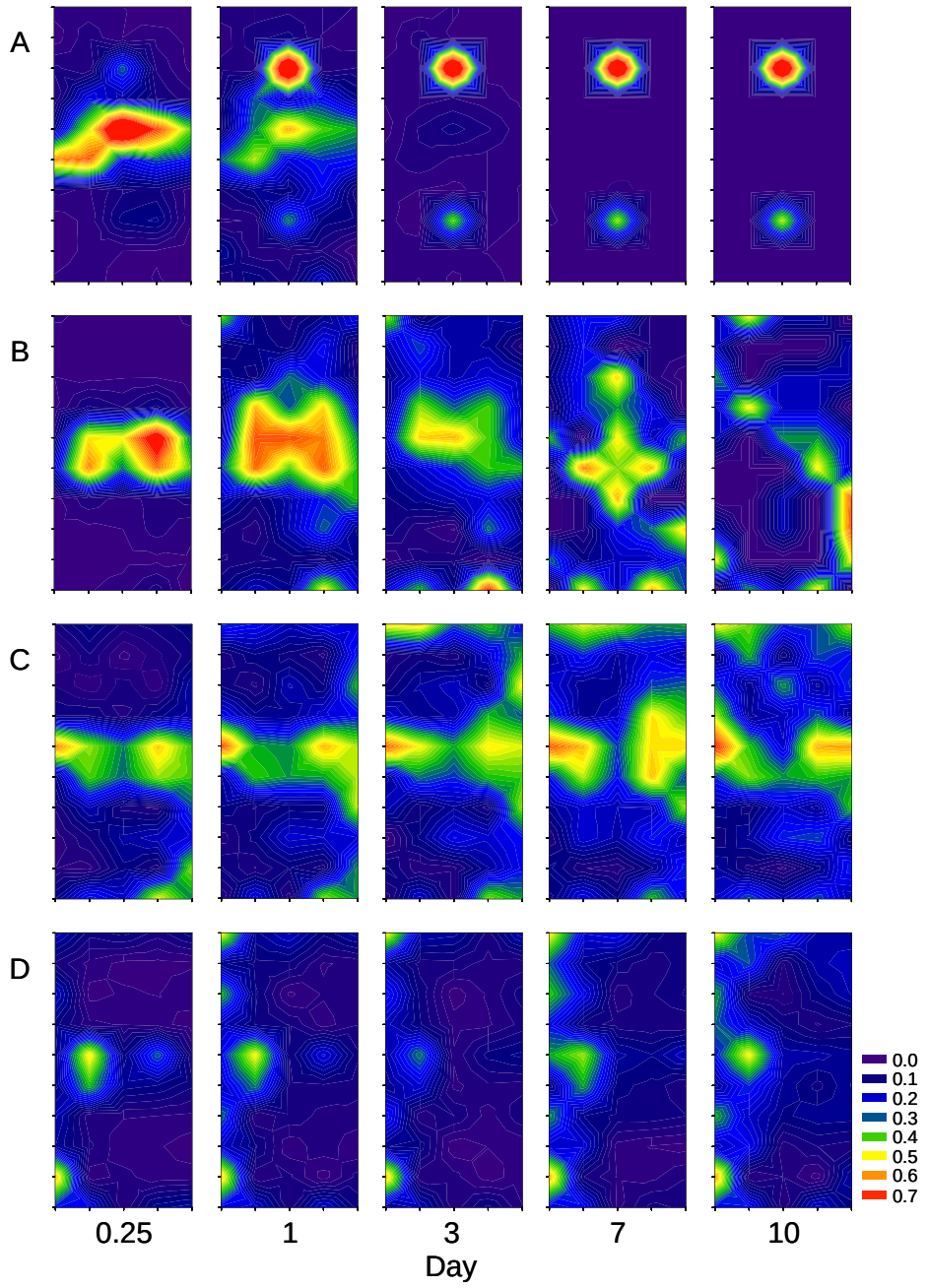

Figure 2.5. Spatial distribution of adult whiteflies on the plants over time in the trap crop trial. *Trialeurodes vaporariorum* in (A) the trap plant treatment and (B) the poinsettia alone treatment. *Bemisia argentifolii* in (C) the trap plant treatment and (D) the poinsettia alone treatment. Note that all maps are based on the same scale; see Figure 2.1 for the experimental design.

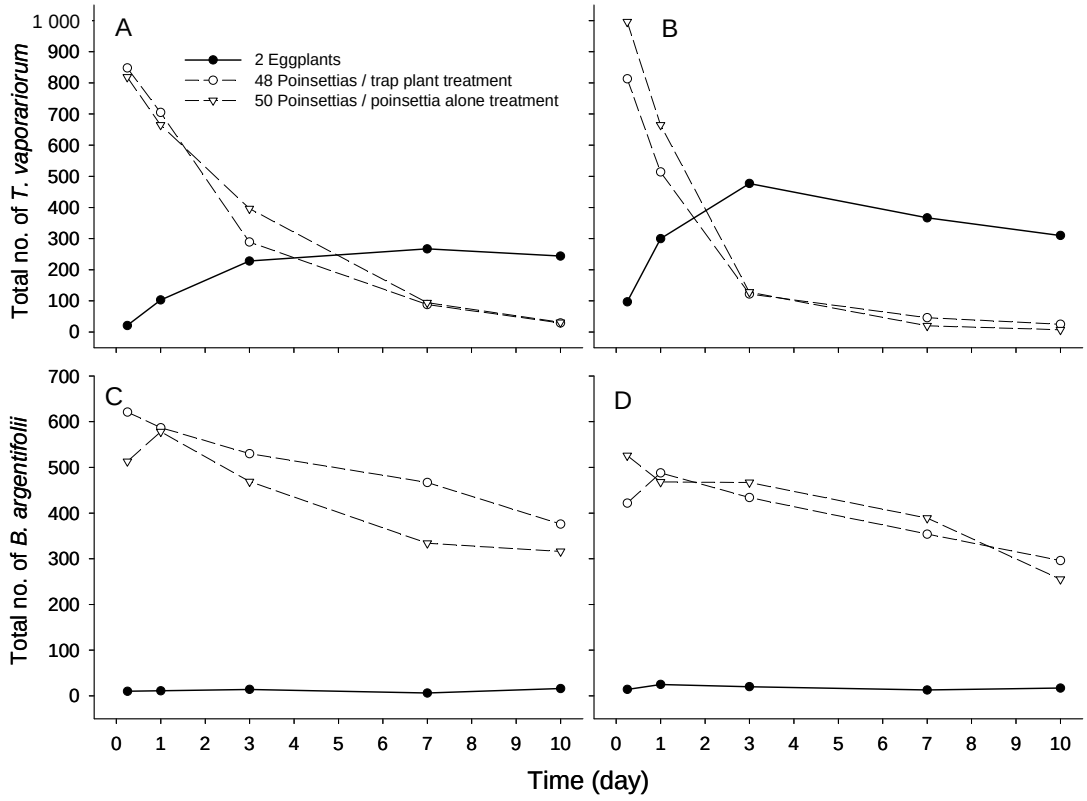

Figure 2.6. Total number of whiteflies observed on eggplants and poinsettias over time in the trap crop trial. *Trialeurodes vaporariorum* in (A) the first trial and (B) the second trial, and *Bemisia argentifolii* in (C) the first trial and (D) the second trial.

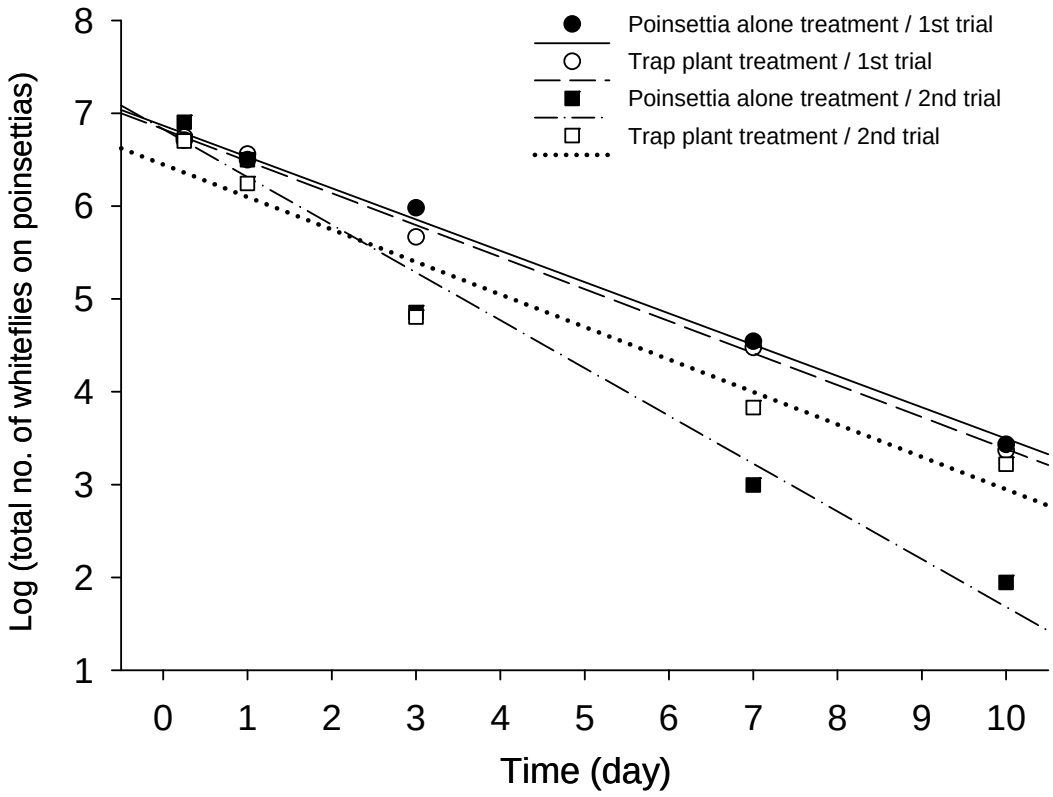

Figure 2.7. Linear regression of log-transformed total number of adult *Trialeurodes vaporariorum* on poinsettias on observation time in the trap crop trial.

Table 2.1. Total number of whitefly nymphs (second or third instars) observed on poinsettias in the trap crop trial. The nymphs were counted 15 days after releasing adults. The sampling unit was five upper leaves of each poinsettia

Whitefly	Trial	Treatment	
		Trap plant (n = 48)	Poinsettia alone (n = 50)
Trialeurodes vaporariorum	First	2 084	1 765
	Second	2 886	1 760
Bemisia argentifolii	First	1 646	1 311
	Second	608	628

Simulation model

We used the model to explore how the mortality rate of adult whiteflies on poinsettia influences the effectiveness of eggplant as a trap crop (Figure 2.2). Here, effectiveness refers to a decrease in the adult density on poinsettia caused by the presence of the eggplant, compared to poinsettia in monoculture. In the simulation, we set the adult mortality rate of whiteflies (m) at 0.1, 0.5, or 0.9 with a constant rate of dispersal (d = 0.5) and a constant attractiveness of eggplant (e = 0.7). The simulation results show that the difference between the two treatments in whitefly density on poinsettia decreases as the mortality of whiteflies increases (Figure 2.8). At the low mortality (m = 0.1), the attractiveness of eggplant (e = 0.7) resulted in a clear difference in the adult densities on poinsettia between the two treatments (Figure 2.8A). However, the density patterns for the two treatments were very similar when the mortality was high (m = 0.9) (Figure 2.8C). The simulation result at the high mortality is very similar to the dynamics of the adult *T. vaporariorum* population in the greenhouse experiment (Figure 2.6A,B).

Discussion

Our cage experiment results suggest that eggplant has limited potential for use as a trap crop in management of *B. argentifolii* on poinsettia, because eggplant was not highly attractive to adults, especially when this whitefly had first settled on poinsettia (Figure 2.4). Our greenhouse-scale experiments support this inference because even when a high density of eggplant was used as a trap crop (1 per 9 m^2, compared to 1 per 100 m^2 used in commercial productions by Murphy, 2007) the eggplant failed to

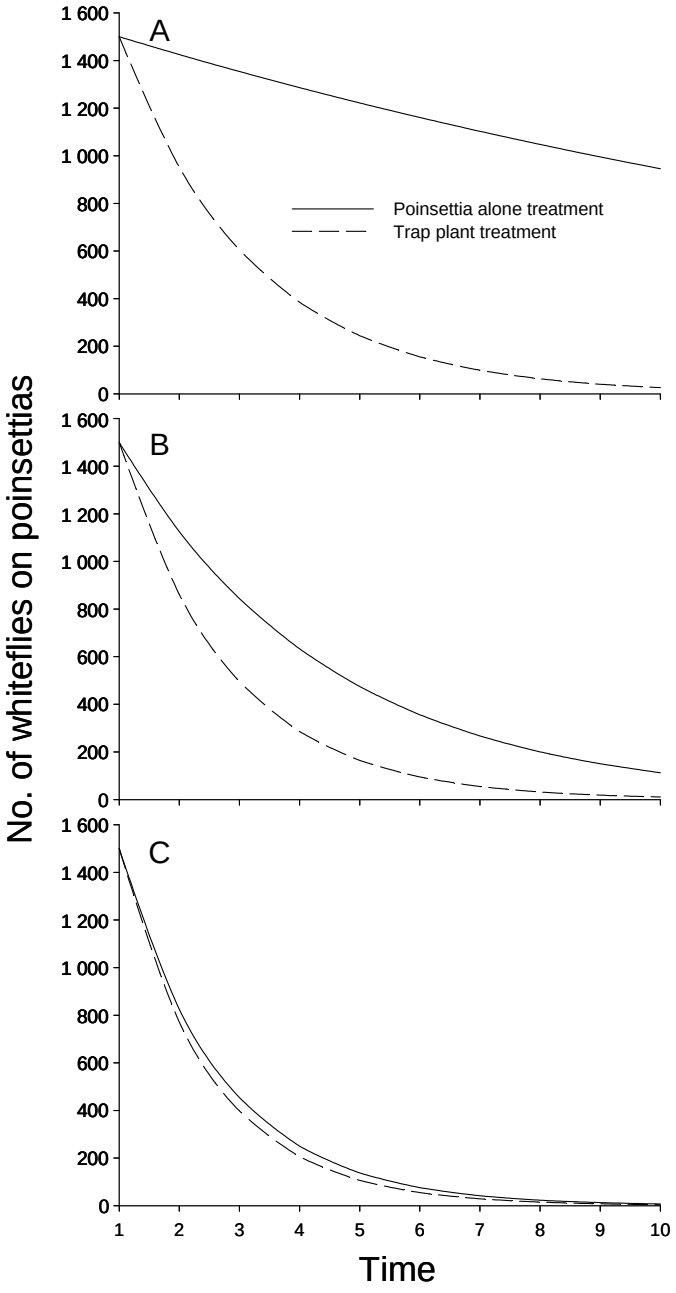

Figure 2.8. Simulation results of the relationship between the adult whitefly mortality on poinsettia and the effectiveness of eggplant as a trap crop. The initial number of adult whiteflies was set at 1 500. The dispersal rate was set at 0.5 and the attraction of whiteflies to eggplant was set at 0.7. The mortality rate was set at (A) 0.1, (B) 0.5, or (C) 0.9.

attract and hold *B. argentifolii* adults (Figures 2.5 and 2.6). Bird & Krüger (2006) found that *B. tabaci* females remained mostly stationary on the same plant for hours and movements among different host plants were rare in continuous 9-h behavioral observations. This behavioral observation corroborates well with our data showing that the number of *B. argentifolii* remained constant over time on either eggplant or poinsettia shortly after the release of adults (Figure 2.3B). Therefore, it is likely that adult *B. argentifolii* readily accept poinsettia as a host after probing plant tissues and having done so, does not respond to eggplant-specific visual or olfactory cues.

It would be worthwhile to test the efficacy of a trap crop in combination with the use of a push component that would help repel *B. argentifolii* away from poinsettia. There have been few attempts to combine a push component with trap cropping in whitefly management (Smith et al., 2000). An effective push component would either reduce whiteflies settling on poinsettia or cause dispersal away from poinsettia, thus preventing whiteflies from probing poinsettia tissues. Probing plant tissues after landing is known as an important step for host plant or plant part selection by whiteflies (Lei et al., 1998). Repellent visual stimuli such as colored plastic mulches might function as a push if they are compatible with agronomic practices in poinsettia production (Hilje et al., 2001). Adult whiteflies are repelled by UV wavelengths reflected by silver and aluminum pigments on plastic mulch (Stansly & Schuster, 1999). Chemical repellents might also be incorporated with the trap crop system. Recent studies have shown that application of mineral oil (Liang & Liu, 2002) or ginger oil (Zhang et al., 2004) has potential as a repellent for management of *B. argentifolii*. Lastly, although little information is available, natural enemies may serve

as a push component in the trap crop system. *Bemisia tabaci* avoids host cucumber plants that harbor the predatory mite *Typhlodromips swirskii* (Nomikou et al., 2003). This avoidance behavior may result in whiteflies being less stationary on poinsettia when natural enemies are present, thus encouraging dispersal. There is a good inventory of commercially available natural enemies for whiteflies and therefore anti-predator/parasitoid behaviors exhibited by whiteflies could be explored further.

Because whiteflies are weak fliers, it is notable that the presence of eggplant dramatically changed the spatial distribution of adult *T. vaporariorum* by attracting and retaining the adults within a relatively short time following release (Figure 2.5A,B). This result is in accordance with our expectation that the greenhouse would provide an advantageous environment for whiteflies to make directed flights to a more preferred host plant. There was virtually no air-flow (ventilation fans off), or there was at most a mild air-flow (ventilation fans on; 0–100 cm/s) in the greenhouse used in our experiments. In contrast, adult whiteflies in open fields are reported to move with air currents from plant to plant until they find an acceptable host (Byrne & Bellows, 1991). This process involves a strategy of downwind displacement on the air currents until oriented flight toward an attractive stimulus can be achieved (Isaacs et al., 1999). The passive displacement of whiteflies is expected to be more prevalent in open fields compared to greenhouses and would likely lengthen the time required for whiteflies to reach a trap crop.

The strong attractiveness of eggplant to adult *T. vaporariorum* led us to predict that eggplant would function as a trap crop for this whitefly on poinsettia. However, our results showed no indication that the presence of eggplant consistently reduced the

number of adult *T. vaporariorum* on poinsettias. That is, the number of the adults decreased on poinsettias in monoculture as fast as the number of adults decreased on poinsettias under the trap cropping (Figures 2.6 and 2.7). We hypothesized that a high mortality of adult *T. vaporariorum* on poinsettia caused the observed pattern, diluting the effectiveness of eggplant as a trap crop. Indeed, our data indicate that adult *T. vaporariorum* suffered from high mortality on poinsettia: adult *T. vaporariorum* decreased by 82% over 3 days in the cage when provided only poinsettias, whereas most of the adults moved away from poinsettia and survived on eggplant if an eggplant was provided (Figure 2.4). A similar pattern was observed in the poinsettia alone treatment of the greenhouse experiment (Figure 2.6A,B). In such a situation, an extra decrease in adult density on poinsettia due to the trap cropping will not be observed because most of the whiteflies found on eggplant would have died on poinsettia if eggplant was not provided. It is not clear what factors were responsible for the observed high mortality of adult *T. vaporariorum* on poinsettia. Given that *T. vaporariorum* has been reported as one of the major whitefly pests in commercial poinsettia crops (Van Driesche et al., 1999, 2001; Murphy, 2007), the high mortality observed in this study was unexpected. One possible explanation is that our test population of *T. vaporariorum* had lost genetic variability through inbreeding, which might limit flexibility as a generalist. The limited plasticity may explain in part the high mortality of adult *T. vaporariorum* on poinsettia, a less-preferred host plant compared to eggplant. The *T. vaporariorum* had been maintained exclusively on bean for more than 20 years. Assuming the limitation of our test population, the potential of eggplant as a trap crop for *T. vaporariorum* cannot be unequivocally determined from

our experimental data.

The simulation study supports our hypothesis that mortality of adult whiteflies on poinsettia influences the effectiveness of the trap crop (Figure 2.8). As mortality increased in the simulations, the density dynamics of adults on poinsettias in monoculture became similar to that under trap cropping. That is, there was almost no change in the density dynamics on poinsettias under trap cropping as mortality of whiteflies changed; however, the density dynamics in monoculture was greatly affected by the mortality rate. Simulated densities with a high mortality rate (Figure 2.8C) are similar to those observed for adult *T. vaporariorum* in the greenhouse experiment (Figure 2.6A,B). In contrast, simulation with a low mortality rate on poinsettia resulted in a density pattern that would indicate trap cropping was effective (Figure 2.8A). This implies that a relatively low mortality of adult whiteflies on poinsettia is a requisite for a highly preferred host plant to function as a trap crop.

In addition, more *T. vaporariorum* nymphs were observed on poinsettias under the trap cropping, compared to poinsettias in monoculture (Table 2.1). The difference was mainly due to a couple of hotspots of the nymphs on poinsettias neighboring the eggplant (data not shown). This suggests that the aggregated adults on the eggplants caused increased oviposition on the neighboring poinsettias. Similar patterns have been identified as one of the risk factors when implementing trap cropping (Hilje et al., 2001).

Because *T. vaporariorum* is a generalist, the performance of this insect on a given plant is strongly affected by prior host plant experience (Van Lenteren & Noldus, 1990). Therefore, we expect that the host plant history of a whitefly

population will play a role in determining their mortality rate on poinsettia and subsequently, the effectiveness of a trap crop. This suggests that the efficacy of trap cropping can vary over a growing season according to the extent to which a *T. vaporariorum* population adapts to poinsettia. Further study is needed to discern how *T. vaporariorum* populations adapt to poinsettia and how this adaptation influences preference for eggplant and the effectiveness of eggplant as a trap crop.

Our work was motivated by the need to develop a common trap crop for management of the two whitefly species. We found that the potential of eggplant as a trap crop must be interpreted differently for *T. vaporariorum* and *B. argentifolii*, because we observed clear differences in the preference for eggplant by these two species. Eggplant was not highly attractive to *B. argentifolii*, and therefore failed to draw a sufficient number of adults away from poinsettia to function as a trap crop. More interestingly, high mortality of adult *T. vaporariorum* diluted the effectiveness of eggplant as a trap crop even when a large number of adults were attracted to the eggplant. This finding warrants further study to investigate the effectiveness of trap cropping as a function of host adaptation, host performance, and host preference of whiteflies. This will allow us to better understand the possible underlying mechanisms governing the efficacy of trap cropping.

REFERENCES

Al-Musa A (1982) Incidence, economic importance, and control of tomato yellow leaf curl in Jordan. Plant Disease 66: 561–563.

Bernays EA (1999) When host choice is a problem for a generalist herbivore: experiments with the whitefly, *Bemisia tabaci*. Ecological Entomology 24: 260–267.

Bird TL & Krüger K (2006) Response of the polyphagous whitefly *Bemisia tabaci* B-biotype (Hemiptera: Aleyrodidae) to crop diversification – influence of multiple sensory stimuli on activity and fecundity. Bulletin of Entomological Research 96: 15–23.

Byrne DN & Bellows TS (1991) Whitefly biology. Annual Review of Entomology 36: 431–457.

Byrne DN, Buchmann SL & Spangler HG (1988) Relationship between wing loading, wingbeat frequency and body mass in homopterous insects. Journal of Experimental Biology 135: 9–23.

Castle SJ (2006) Concentration and management of *Bemisia tabaci* in cantaloupe as a trap crop for cotton. Crop Protection 25: 574–584.

Ellsworth PC, Chernicky JP, Byrne DN, Gibson R & Meade D (1992) A native weed as a trap crop for whiteflies in cotton. Proceedings Beltwide Cotton Insect Research and Control Conference (ed. by DJ Herber), pp. 911–913. National Cotton Council, Nashville, TN, USA.

Faria M & Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. Crop

Protection 20: 767–778.

Gerling D, Alomar O & Arnó J (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Protection 20: 779–799.

Greenberg SM, Jones WA & Liu T-X (2002) Interactions among two species of *Eretmocerus* (Hymenoptera: Aphelinidae), two species of whiteflies (Homoptera: Aleyrodidae), and tomato. Environmental Entomology 31: 397–402.

Heinz KM & Parrella MP (1994) Poinsettia (*Euphorbia pulcherrima* Willd. ex Koltz.) cultivar-mediated difference in performance of five natural enemies of *Bemisia argentifolii* Bellows and Perring, n. sp. (Homoptera: Aleyrodidae). Biological Control 4: 305–318.

Hilje L, Costa HS & Stansly PA (2001) Cultural practices for managing *Bemisia tabaci* and associated viral diseases. Crop Protection 20: 801–812.

Hokkanen HM (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119–138.

Isaacs R, Willis MA & Byrne DN (1999) Modulation of whitefly take-off and flight orientation by wind speed and visual cues. Physiological Entomology 24: 311–318.

Lei H, Tjallingii WF & Van Lenteren JC (1998) Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. Entomologia Experimentalis et Applicata 88: 73–80.

Liang G & Liu T-X (2002) Repellency of a kaolin particle film, surround, and a mineral oil, sunspray oil, to silverleaf whitefly (Homoptera: Aleyrodidae) on melon in the laboratory. Journal of Economic Entomology 95: 317–324.

McAuslane HJ, Johnson FA, Colvin DL & Sojack B (1995) Influence of foliar

pubescence on abundance and parasitism of *Bemisia argentifolii* on soybean and peanut. Environmental Entomology 24: 1135–1143.

Murphy G (2007) Biocontrol of whitefly in poinsettia using trap plants. Bio-Bulletin. http://207.5.17.151/biobest/en/bulletin/Biocontrolofwhiteflypoinsettia.htm

Naranjo SE (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop Protection 20: 835–852.

Nomikou M, Janssen A & Sabelis MW (2003) Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. Oecologia 136: 484–488.

Perring TM, Mayberry KS & Natwick ET (1995) Silverleaf whitefly management in cauliflower using a trap crop. Silverleaf Whitefly: 1995 Supplement to the 5-year National Research and Action Plan (ed. by TJ Henneberry, NC Toscano, RM Faust & JR Coppedge), p. 151. USDA-ARS, Washington, DC, USA.

Schuster DJ (2004) Squash as a trap crop to protect tomato from whitefly-vectored tomato yellow leaf curl. International Journal of Pest Management 50: 281–284.

Shelton AM & Badenes-Perez FR (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Smith HA, Koenig RL, McAuslane HJ & McSorley R (2000) Effect of silver reflective mulch and a summer squash trap crop on densities of immature *Bemisia argentifolii* (Homoptera: Aleyrodidae) on organic bean. Journal of Economic Entomology 93: 726–731.

Smith HA & McSorley R (2000) Potential of field corn as a barrier crop and eggplant as a trap crop for management of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on

common bean in north Florida. Florida Entomologist 83: 145–158.

Stansly PA & Schuster DJ (1999) Impact of mulch color and reflectivity on yield and pest incidence. Citrus and Vegetable Magazine 63: 9–12.

Stevens TJ, Kilmer RL & Glenn SJ (2000) An economic comparison of biological and conventional control strategies for whiteflies (Homoptera: Aleyrodidae) in greenhouse poinsettias. Journal of Economic Entomology 93: 623–629.

Tsai JH & Wang K (1996) Development and reproduction of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on five host plants. Environmental Entomology 25: 810–816.

Vandermeer J (1989) The Ecology of Intercropping. Cambridge University Press, Cambridge, UK.

Van Driesche RG, Hoddle MS, Lyon S & Sanderson JP (2001) Compatibility of insect growth regulators with *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control on poinsettia. Biological Control 20: 132–146.

Van Driesche RG, Lyon SM, Hoddle MS, Roy S & Sanderson JP (1999) Assessment of cost and performance of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control in commercial poinsettia crops. Florida Entomologist 82: 570–594.

Van Lenteren JC & Noldus LPJJ (1990) Whitefly-plant relationships: behavioral and ecological aspects of whitefly-plant relationships. Whiteflies: Bionomics, Pest Status and Management (ed. by D Gerling), pp. 47–89. Intercept, Andover, UK.

Zhang W, McAuslane HJ & Schuster DJ (2004) Repellency of ginger oil to *Bemisia*

argentifolii (Homoptera: Aleyrodidae) on tomato. Journal of Economic Entomology 97: 1310–1318.

CHAPTER 3*

EFFECT OF HOST EXPERIENCE OF THE GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM*, ON TRAP CROPPING EFFECTIVENESS

This chapter was published in Entomologia Experimentalis et Applicata 137: 193-203 (2010).

Abstract

This study evaluated whether experience of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) on a poinsettia cash crop, *Euphorbia pulcherrima* Willd. ex Koltz (Euphorbiaceae), influences the effectiveness of an eggplant trap crop, *Solanum melongena* L. (Solanaceae). Two whitefly strains were tested: one was reared on poinsettia (poinsettia-strain) and a second was reared on bean [*Phaseolus vulgaris* L. (Fabaceae)] (bean-strain). We first determined whether host experience altered the preference of adult whiteflies for eggplant and their survivorship on poinsettia. Then, we determined whether changes in the preference and/or survivorship influenced the effectiveness of the trap cropping. Adult whiteflies from both strains consistently redistributed and settled on an eggplant trap crop in significantly higher numbers compared to poinsettia. The adult survivorship of the poinsettia-strain whiteflies was slightly higher on poinsettia than on the bean-strain. In research greenhouse experiments, we found that the trap cropping consistently resulted in a decrease in the density of the poinsettia-strain whiteflies on the cash crop compared to that in monoculture. However, higher adult whitefly survivorship on eggplant than on poinsettia could compromise its effectiveness as a trap crop in poinsettia. The

effectiveness of trap cropping, as reflected by the whitefly density reduction on a poinsettia cash crop, was significantly smaller than the attractiveness of the trap crop, as indicated by the whitefly abundance on an eggplant trap crop.

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is a serious insect pest that feeds on numerous cultivated and uncultivated plants (Byrne & Bellows, 1991). This polyphagous herbivore has variable performance on and preference for a variety of host plants. Preference and performance are correlated and have been determined for several host plants: eggplant (Solanaceae) > gherkin (Cucurbitaceae) > cucumber (Cucurbitaceae)> gerbera (Asteraceae) > melon (Cucurbitaceae) > tomato (Solanaceae) > sweet pepper (Solanaceae) (van Lenteren & Noldus, 1990). Hierarchical host use by *T. vaporariorum* might be used to develop trap crop systems in which a preferred host plant is deployed to attract pests away from a less-preferred cash crop (Hokkanen, 1991; Hilje et al., 2001; Shelton & Badenes-Perez, 2006). This cultural management practice has the potential to offer significant economic and environmental benefits and might be integrated with other management programs.

For *Bemisia* whiteflies, experiments with trap crop systems have yielded mixed results. Some authors found that trap cropping was effective at reducing whitefly density or whitefly-vectored viruses on cash crops (Al-Musa, 1982; Ellsworth et al., 1992; Schuster, 2004; Castle, 2006), whereas others have concluded that trap cropping did not consistently reduce whitefly density (McAuslane et al., 1995; Perring et al.,

1995; Smith & McSorley, 2000; Smith et al., 2000). Although *Bemisia* whiteflies and *T. vaporariorum* co-occur in many productions (Greenberg et al., 2002), there have been few attempts to evaluate the potential of trap cropping for *T. vaporariorum*. To date, eggplant (*Solanum melongena* L.) has been tested as a trap crop for *T. vaporariorum* on greenhouse poinsettia [*Euphorbia pulcherrima* Willd. ex Koltz (Euphorbiaceae)] in commercial greenhouses (Murphy et al., 2007) and via manipulative experiments (Lee et al., 2009). These studies reported in common that eggplants attracted a large number of adult *T. vaporariorum*, indicating that the use of eggplants might have potential as a trap crop system for *T. vaporariorum*.

Plant traits such as plant sap, secondary metabolites, and leaf morphology are thought to contribute to the hierarchical host acceptance by whiteflies (Noldus et al., 1986; van Vianen et al., 1988; van Lenteren & de Ponti, 1990). Whiteflies are also known to show different levels of performance on and preference for a given plant depending on host experience [see van Lenteren & Noldus (1990) for review]. In general, *T. vaporariorum* performance on a host plant improves as generations from a population are produced on a particular host. Indeed, *T. vaporariorum* can, within a few generations, improve performance on a host plant for which a population has had no experience in the recent past (van Boxtel, 1980; Dorsman & van de Vrie; 1987; Thomas, 1993a). It is not clear whether this reported plasticity is due to phenotypic or genotypic changes in whitefly populations.

The rapid, flexible host switch by *T. vaporariorum* should be taken into account when developing and implementing trap crop systems because the plasticity can alter the performance of whiteflies on a cash crop and their preference for a trap

crop. However, little information is available about how host acclimation on the cash crop by generalist pests such as whiteflies can influence the effectiveness of trap cropping, even though such adjustment may occur over a growing season. For example, a *T. vaporariorum* population might perform poorly on a new cash crop upon migrating from their original host but, within a growing season, their performance on and preference for the cash crop could be enhanced. It is also possible for a whitefly population to perform relatively well on the cash crop from an early infestation stage, especially when whiteflies dispersed from neighboring productions of the same crop (e.g., open field vegetables) or they were transported from nursery plants (e.g., greenhouse floricultures). These alterations by whiteflies on the cash crop are expected to result in a reduced attraction of the whiteflies to a trap crop. In addition, there are local strains of *T. vaporariorum* that reflect a long relationship between whitefly populations and their regional host plants (Thomas, 1993b). This variation may result in different pre-conditionings of whiteflies which can facilitate or hamper their acclimation to a given cash crop.

Previously, we tested eggplant for use as a trap crop for *T. vaporariorum* on greenhouse poinsettia (Lee et al., 2009). In the experiments, we used a *T. vaporariorum* strain that had no experience on either poinsettia or eggplant and found an unexpected result: although adult whiteflies were strongly attracted to eggplants, there was no indication that, compared to whitefly density in poinsettia monoculture, the presence of eggplant trap crop consistently reduced the whitefly density on poinsettias. We inferred that the observed pattern was in part due to low survivorship of adult *T. vaporariorum* on poinsettias, which caused the adult whitefly density on

the cash crop in monoculture to decrease as fast as that under trap cropping. In contrast, adult whiteflies showed a high survivorship on eggplant in the experiments. As a result, a majority of adult whiteflies found on eggplants were simply saved from death by dispersing from the marginally-suitable cash crop to the highly-suitable trap crop. A simulation model supported this inference and was used to show that a relatively low mortality of adult whiteflies on a cash crop is a requisite for a highly-attractive host to function as an effective trap crop.

This finding led us to investigate whether experience of *T. vaporariorum* on poinsettia can alter the effectiveness of eggplant as a trap crop. Experience of whiteflies on poinsettia is predicted to enhance their performance including survivorship on a poinsettia cash crop and concurrently decrease their preference for an eggplant trap crop. The trade-off would influence the effectiveness of trap cropping by changing whitefly density dynamics both in monoculture and under trap cropping. In this study, we tested this hypothesis using two whitefly strains with different rearing histories: one strain was reared on bean [*Phaseolus vulgaris* L. (Fabaceae)], which represents a newly-colonizing population on poinsettia, and a second strain was reared on poinsettia, which represents a reproducing population on the cash crop. We first measured the preference by the two whitefly strains for eggplant vs. poinsettia and their survivorship on poinsettia. We then examined how changes in the preference and/or survivorship influenced the effectiveness of trap cropping.

Materials and methods

Insects and plants

An original *T. vaporariorum* culture, referred to as the 'bean-strain', was maintained on bean (*P. vulgaris*) for >10 years in a walk-in growth chamber. A new whitefly culture, referred to as the 'poinsettia-strain', was started on poinsettia (*E. pulcherrima*) with several thousands of adult whiteflies collected from the original culture. The poinsettia-strain was reared on poinsettia for >7 months prior to the experiments. The two whitefly colonies were maintained under 20-25 °C and L14:D10 photoperiod. Poinsettias (cv. 'Freedom Red') and eggplants (cv. 'Dusky SG') were grown in 15-cm pots with Pro-Mix soil (Premier Horticulture, Quakertown, PA, USA) and fertilized 5 days a week. Plants were not treated with any pesticides and were inspected for whitefly infestations before use in the experiments. The plants were grown in greenhouses under 20-25 °C and L14:D10 photoperiod.

Preference of whiteflies for eggplant: cage experiments

Two cage experiments were conducted to measure the preference of adult *T. vaporariorum* for eggplant vs. poinsettia. The first experiment was a 'dual choice' experiment in which whiteflies were released midway between the two plants. The second was a 'sequential choice' experiment, in which whiteflies were allowed to first settle on poinsettia and were then provided with eggplant. In the first experiment, one eggplant (6 weeks old, 33 cm tall) and one poinsettia (7 weeks old, 29 cm tall) were placed 35 cm apart in a BugDorm-4180 cage (48 × 48 × 93 cm; MegaViewScience Education Services, Taichung, Taiwan). The position of the plants was randomly assigned to the left or right side of the cage. Sixty adult whiteflies (mixed age and sex) of each whitefly strain were aspirated separately into 25-ml glass vials, and allowed to

acclimate in the vial for 0.5-1 h. Then, whiteflies of each strain were released separately from the middle of the cage by opening the vial's lid. The number of adult whiteflies on each plant was recorded by carefully lifting up and tilting the plants and counting the insects on the underside of leaves. Observations were made at 3, 24, 48, and 72 h after the release.

In the second experiment, one poinsettia (5 weeks old, 28 cm tall) was placed in a BugDorm-3120 cage (60 × 60 × 60 cm) and 50 adult whiteflies (mixed age and sex) of each strain were released separately in the cages from the bottom of the plant. After 24 h, the number of adult whiteflies that settled on the poinsettia was recorded and the whitefly-infested poinsettia was carefully transferred into another BugDorm-4180 cage (48 × 48 × 93 cm) containing one uninfested eggplant (6 weeks old, 26 cm tall). The number of adult whiteflies on each plant was recorded at 3, 24, 48, and 72 h as described above. The experiments were conducted in a greenhouse under 23-24 °C and L14:D10 photoperiod. The tests were replicated 10 times.

The data were analyzed using repeated measures ANOVA because the number of whiteflies was recorded repeatedly on the same plant in an experimental unit (i.e., cage). The response variable was expressed as the proportion of whiteflies on eggplant at each observation time and was arcsine-transformed to normalize the data. In the ANOVA, the fixed factors were time, whitefly strain, and their interactions; the random factor was a spatial block (distance from a chiller) that was used to minimize the possible effect of a gradient in the greenhouse environment. The linear mixed model was analyzed using Proc MIXED in SAS (SAS Institute, Cary, NC, USA). Covariance structures were selected based on model fit statistics. The total number of

whiteflies observed on the two plants was also compared between the two whitefly strains using repeated measures ANOVA. Within whitefly strain, if the proportion of whiteflies on eggplant varied over time, the proportion at a given observation time was compared to 0.5 to examine whether whiteflies significantly preferred eggplant over poinsettia at that observation.

Preference of whiteflies for eggplant: greenhouse experiment

A greenhouse compartment (8.0 × 6.5 m) was partitioned into two experimental plots by hanging a curtain of Agribon+ AG-19 screen (3.5 m high; Green Mountain Transplants, Arundel, ME, USA). An experimental arena was created in each plot that consisted of one eggplant (6 weeks old, 34 cm tall) and eight poinsettias (7 weeks old, 29 cm tall) placed 50 cm from the eggplant and arranged as an octagon. Thirty adult whiteflies (mixed age and sex) of each strain were collected separately into 25-ml glass vials and then released into separate experimental arenas from the bottom of each poinsettia. Thus, a total of 240 adult whiteflies of each strain were released from the eight poinsettias in the experimental arenas. Observations were made as described above at 3 h after the release, and then every 24 h for 5 days. The experiment was carried out at 22-23 °C and L14:D10 photoperiod. The test was replicated 4 times. The data were analyzed using repeated measures ANOVA as described above with the exception that the blocking factor was date of replication. The response variable was the proportion of whiteflies on the eggplant relative to the total on the nine plants (one eggplant + eight poinsettias) in the experimental plots.

Survivorship of whiteflies on poinsettia

An experiment was conducted to estimate adult survivorship of the two whitefly strains on poinsettia. It has been shown that sweetpotato whiteflies [*Bemisia tabaci* (Gennadius)] cannot survive longer than 24 h without feeding and the adults that have not settled on plants are subject to death within 24 h due to starvation or dehydration (Fenigstein et al., 2001). The same pattern was observed with *T. vaporariorum* when adult whiteflies were deprived of plant feeding (data not shown). Therefore, the number of adult whiteflies observed on a plant is a practical proxy of adult survivorship on the plant when the insects are not confined into a small arena such as a clip cage. Thirty newly-emerged adult whiteflies (<2 days old, approximately 1:1 sex ratio) of each strain were collected into separate 25-ml glass vials and the whiteflies were released in separate BugDorm-3120 cages (60 × 60 × 60 cm) from the bottom of a poinsettia (6 weeks old). The number of adult whiteflies on poinsettia was recorded daily for 15 days as described above. The experiment was conducted in a greenhouse under 23 °C and L14:D10 photoperiod and replicated 10 times. The data were analyzed using repeated measures ANOVA as described above, but the response variable was the number of whiteflies on poinsettia in each cage. If there was a significant interaction between time and whitefly strain, the numbers were also compared between the two whitefly strains using a t-test at each observation time.

Trap cropping trial

A greenhouse experiment was carried out with the poinsettia-strain to determine whether eggplant could be effective as a trap crop for this whitefly strain by reducing

the number of adult whiteflies and their oviposition on poinsettias, compared to that in monoculture. Because our previous study demonstrated that the use of eggplant was not effective for the bean-strain, only the poinsettia-strain was used in this study. To test this, two treatments were created in a research greenhouse: a 'trap plant treatment' with 53 poinsettia + 2 eggplants and a 'poinsettia alone treatment' with 55 poinsettias only. A greenhouse compartment (8.0 × 6.5 m) was partitioned into two experimental plots by hanging a curtain of Agribon+ AG-19 screen (3.5 m high). In the poinsettia alone plot, 55 poinsettias (10 weeks old, 32 cm tall) were placed into a 5 × 11 (column × row) layout on greenhouse benches (3 × 6 m). In the trap plant plot, the two poinsettias at 3–3 (column–row) and 3–9 were replaced with two eggplants (6 weeks old, 42 cm tall). One hundred adult whiteflies (mixed age and sex) were collected from the poinsettia-strain into a 25-ml glass vial and two vials were placed at the base of the five poinsettias located in the middle of the experimental plot (row 6). Thus, a total of 1 000 whiteflies were released in each treatment. The number of adult whiteflies was recorded on every plant as described above after 5 h and then every 2 days for 11 days. At the end of the experiment, three upper leaves were randomly collected from each plant and examined under a microscope to record the number of whitefly eggs. The experiment was conducted under 22-23 °C and L14:D10 photoperiod, and was replicated three times.

The spatial distribution of adult whiteflies was visualized via contour maps to examine how the presence of eggplant trap crop altered the distributions of adults over time (SigmaPlot 7.0, SPSS). To draw the contour maps, counts of adult whiteflies on each plant were standardized by dividing each data point by the maximum count on

the plants at each observation time. Following standardization, the three data points (i.e., three replications) for each spatial coordinate were averaged. These average values were used to create contour maps for each observation time. To examine the effect of trap cropping on whitefly density on the cash crop, the effectiveness of trap cropping was calculated as the number of adult whiteflies on poinsettias in the trap crop treatment minus that in the poinsettia alone treatment. Then, the effectiveness of the trap cropping was compared to the attractiveness of the trap crop which was represented by the number of whiteflies on the eggplants. The numbers of whitefly eggs on poinsettias were compared between the two treatments by first scaling the counts in the three replicates to a common 0-1 scale and then determining whether the difference between the two treatments exceeded zero using a one-sided t-test (JMP 8.0, SAS).

Results

Preference of whiteflies for eggplant: cage experiments

When the two strains of adult *T. vaporariorum* were given a choice between poinsettia and eggplant, both whitefly strains settled on eggplant in significantly higher numbers than on poinsettia (bean-strain: $t = 12.93$, d.f. = 16, $P<0.0001$; poinsettia-strain: $t = 8.38$, d.f. = 16, $P<0.0001$) (Figure 3.1A,B). The preference by the bean-strain for eggplant was significantly greater than that of the poinsettia-strain ($F_{1,16} = 6.99$, $P = 0.018$); however, the accumulation pattern of adult whiteflies on eggplant over time was similar for the two strains ($F_{3,54} = 1.59$, $P = 0.20$). More adult whiteflies (>75%) were observed on eggplant compared to poinsettia commencing with the 3-h

observation (bean-strain: t = 13.18, d.f. = 9, P<0.0001; poinsettia-strain: t = 3.04, d.f. = 9, P = 0.0071) and the proportion of adults on eggplant increased by 9% for both strains over 3 days ($F_{3,54}$ = 13.58, P<0.0001). The total number of adult whiteflies observed on the two plants remained constant over time for the bean-strain ($F_{3,27}$ = 0.17, P = 0.92), whereas the total number of the poinsettia-strain decreased slightly by 2.20 after 3 days ($F_{3,27}$ = 5.55, P = 0.0042) (Figure 3.1A,B).

In the second experiment where adult whiteflies were allowed to first settle on poinsettia and were then provided with eggplant, both whitefly strains moved from poinsettia to eggplant over the course of the study (bean-strain: $F_{3,27}$ = 137.56, P<0.0001; poinsettia-strain: $F_{3,27}$ = 69.24, P<0.0001) (Figure 3.1C,D). However, the bean-strain adults moved from poinsettia to eggplant faster than the poinsettia-strain did ($F_{3,54}$ = 3.95, P = 0.013). The proportion of the bean-strain adults on eggplant exceeded 50% at the 24-h observation (t = 3.80, d.f. = 9, P = 0.0021), whereas the poinsettia-strain did so after 3 days (t = 2.26, d.f. = 9, P = 0.025). As a result, the preference by the bean-strain for eggplant was significantly greater than that of the poinsettia-strain ($F_{1,18}$ = 70.36, P<0.0001). For both whitefly strains, the total number of adults observed on the two plants remained constant over time (bean-strain: $F_{3,27}$ = 1.69, P = 0.19; poinsettia-strain: $F_{3,27}$ = 2.51, P = 0.080) (Figure 3.1C,D).

Preference of whiteflies for eggplant: greenhouse experiment

Three hours following the release of adult whiteflies, 84% of both strains were recovered on the plants (one eggplant + eight poinsettias) and of those on plants, more than 95% were on poinsettias (Figure 3.2).

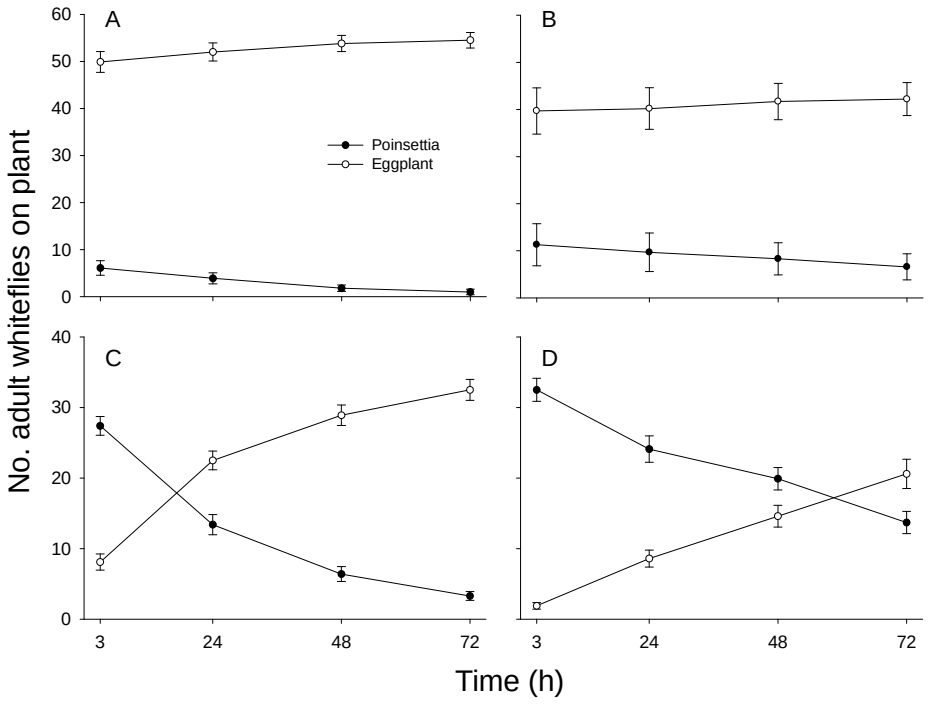

Figure 3.1. Mean number (± SEM) of adult whiteflies observed on eggplant or poinsettia over time when given a choice between the two plants in a cage. (A) Bean-strain and (B) poinsettia-strain in the dual choice experiment; (C) bean-strain and (D) poinsettia-strain in the sequential choice experiment.

Thereafter, whiteflies of both strains continuously moved from poinsettias to eggplant (bean-strain: $F_{5,15} = 456.84$, $P<0.0001$; poinsettia-strain: $F_{5,15} = 67.51$, $P<0.0001$) resulting in 93% of the bean-strain and 87% of the poinsettia-strain observed on eggplant after 5 days. Bean-strain adults moved more quickly to eggplant, compared to poinsettia strain adults ($F_{5,30} = 3.74$, $P = 0.0095$). Therefore, the proportion of the bean-strain adults on eggplant was significantly greater than that of the poinsettia-strain over time ($F_{1,6} = 25.79$, $P = 0.0023$) (Figure 3.2A). However, the difference in the proportions between the two strains decreased from 20 to 6% during the study and was marginally significant at the end of the experiment ($F_{1,5} = 6.51$, $P = 0.051$). That is, the expression of host preference by the two strains for eggplant was similar after 5 days. The total number of adults observed on the nine plants decreased over time for both whitefly strains ($F_{5,30} = 18.73$, $P<0.0001$), and the numbers were not significantly different between the two strains ($F_{1,6} = 3.83$, $P = 0.098$) (Figure 3.2B).

Survivorship of whiteflies on poinsettia

Both whitefly strains showed an exponential decrease in the number of adult whiteflies on poinsettia over time, but the number of the bean-strain adults decreased faster than the poinsettia-strain did ($F_{14,252} = 1.81$, $P = 0.038$) (Figure 3.3). Accordingly, a greater number of the poinsettia-strain adults were observed on poinsettia compared to the bean-strain ($F_{1,18} = 4.45$, $P = 0.049$). The number of adults on poinsettia was not significantly different between the two whitefly strains at any observations during the first 9 days, but the difference was significant thereafter (t-test: $P<0.05$).

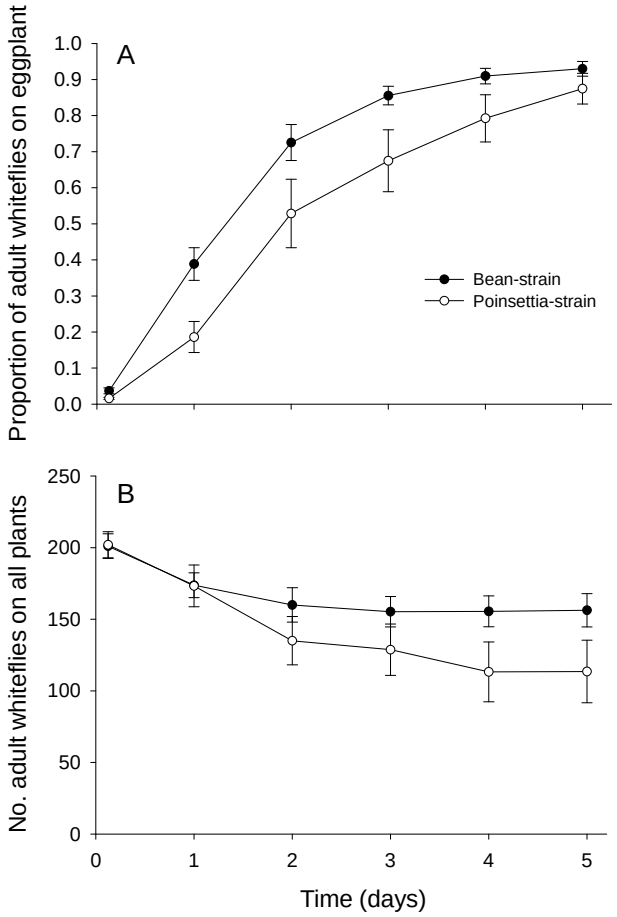

Figure 3.2. (A) Mean proportion (± SEM) of adult whiteflies observed on one eggplant surrounded by eight poinsettias over time in the greenhouse experiment. (B) Mean number (± SEM) of total adult whiteflies observed on the nine plants over time in an experimental arena.

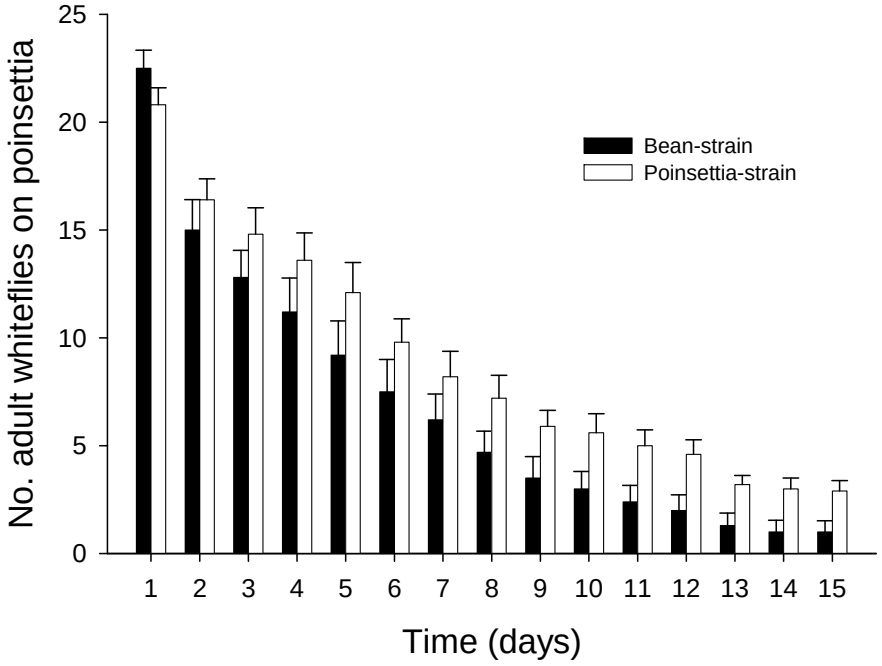

Figure 3.3. Mean number (+ SEM) of adult whiteflies observed on poinsettia over time in the survivorship experiment. Thirty newly-emerged adults were released in each cage at the beginning of the experiment.

Trap cropping trial

The two eggplants in the trap plant treatment attracted many adult whiteflies within 3 days and retained adults throughout the study. By contrast, a large number of adult whiteflies in the poinsettia alone treatment remained aggregated on the poinsettias onto which they were released (Figure 3.4). The high and rapid attraction of adult whiteflies to the eggplants resulted in a clear difference in the spatial distributions of adults between the two treatments after 3 days. The attraction of whiteflies to eggplant reduced the number of whiteflies on poinsettias, compared to poinsettias in monoculture, in all three trials (Figure 3.5); however, the accumulations of whiteflies on eggplants beyond 24 h did not produce an extra decrease in the number of whiteflies on poinsettias in the trap plant treatment compared to that in monoculture. That is, the difference in the numbers of whiteflies on poinsettias between the two treatments did not increase reciprocally with the accumulating number of whiteflies on eggplants (Figure 3.5). As a result, the effectiveness of the trap cropping, as reflected by actual reductions in whitefly abundance on the cash crop, was always smaller than the attractiveness of trap crop, as reflected by whitefly abundance on the trap crop, except for the first 24 h (Figures 3.5 and 3.6). The trap cropping significantly reduced the total number of whitefly eggs on poinsettias by 15-52% compared to that in monoculture ($t = 3.20$, d.f. = 2, $P = 0.043$) (Figure 3.7).

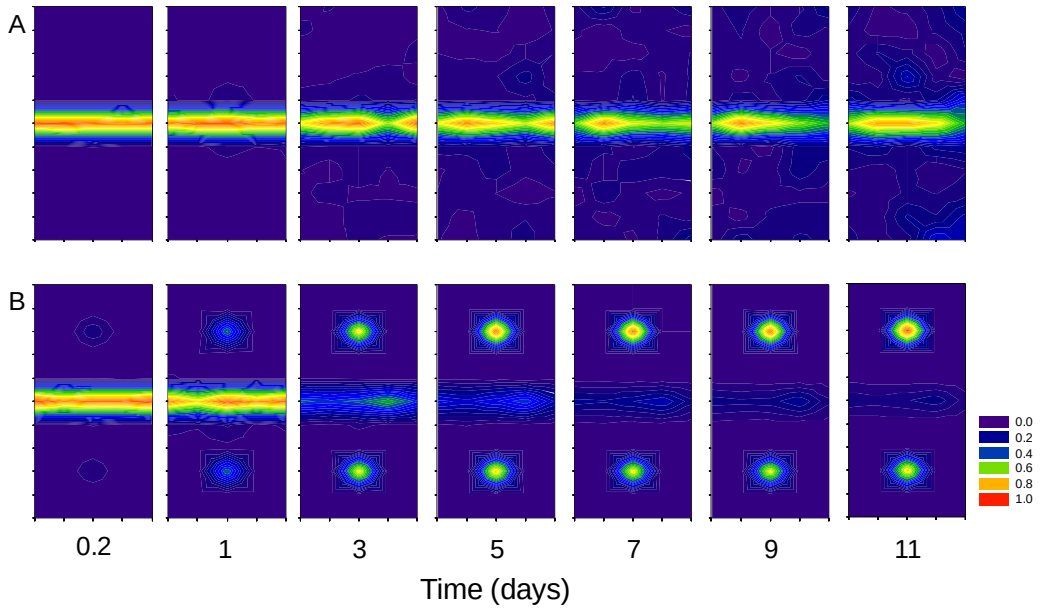

Figure 3.4. Contour maps of spatial distributions of the poinsettia-strain adults observed on the plants over time in the trap cropping trial. (A) Poinsettia alone treatment and (B) trap plant treatment. Counts of adult whiteflies on each plant were standardized to a 0-1 scale by dividing each data point by the maximum count on the plants at each observation time. Following standardization, the three data points (i.e., three replications) for each spatial coordinate were averaged.

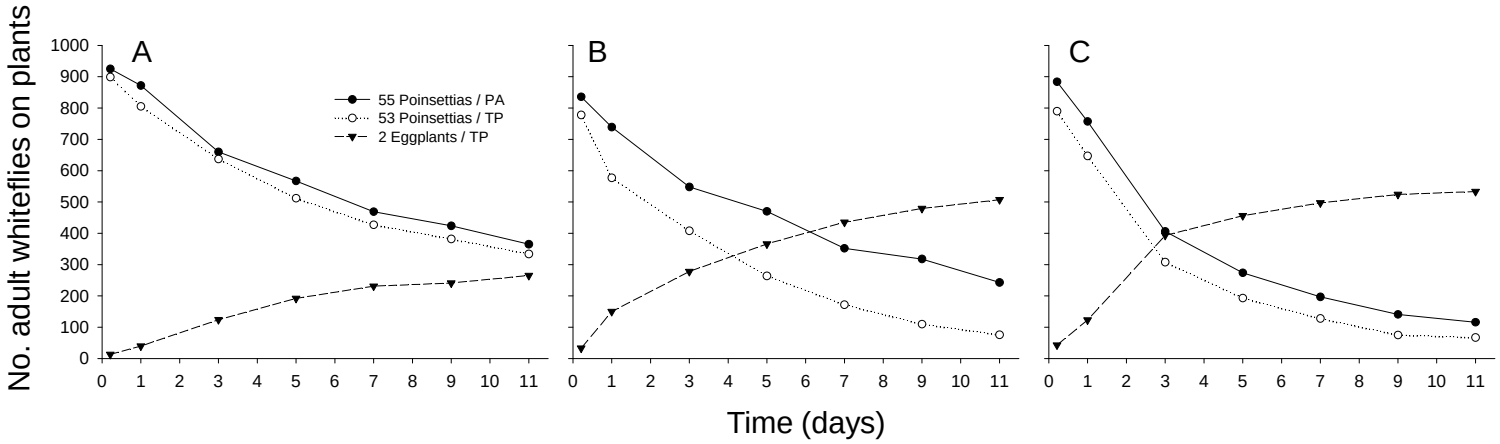

Figure 3.5. Total number of adult whiteflies observed on eggplants and poinsettias over time in the (A) first, (B) second, and (C) third trap cropping trials. PA, poinsettia alone treatment; TP, trap plant treatment.

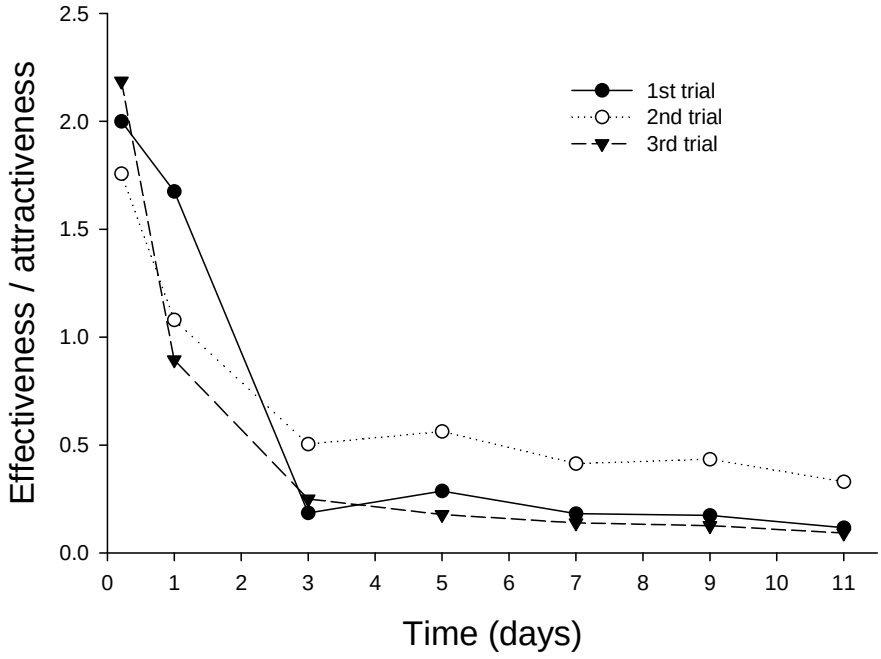

Figure 3.6. Ratio of the effectiveness of trap cropping over the attractiveness of the trap crop over time in the three trap cropping trials. The effectiveness of trap cropping was calculated as the total number of adult whiteflies on poinsettias in the poinsettia alone treatment minus the total number in the trap plant treatment. The attractiveness of the trap crop was the total number of adult whiteflies on eggplants.

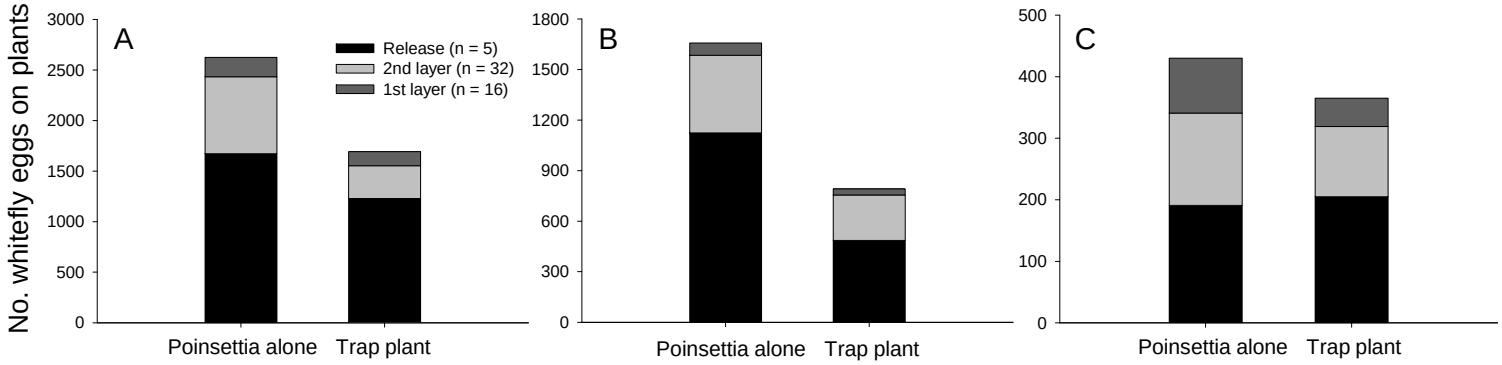

Figure 3.7. Total number of whitefly eggs on poinsettias in the (A) first, (B) second, and (C) third trap cropping trials. The sampling unit was three upper leaves of each poinsettia. 'Release' denotes the five poinsettias from which adult whiteflies were released; '1st layer' refers to the 16 poinsettias neighboring two eggplants in the trap plant treatment and 16 poinsettias at the same location in the poinsettia alone treatment; '2nd layer' refers to the outer layer of 32 poinsettias neighboring the 1st layer.

Discussion

When bean-reared and poinsettia-reared strains of adult *T. vaporariorum* were given a choice between poinsettia and eggplant in cages, both strains settled on eggplant in significantly higher numbers than on poinsettia. For both strains, significantly more whiteflies were observed on eggplant 3 h following the release of adults and thereafter. This indicates that adult whiteflies detected and responded to eggplant cues shortly after being provided a choice between the two plants. The plant-originated stimuli, which are thought to induce the observed fast attraction of whiteflies to eggplant, might include visual and olfactory cues; vision is a dominant sensory modality employed by whiteflies in host plant location. Previous studies have demonstrated that *T. vaporariorum* are attracted to the green part of the visual spectrum (approximately 550 nm) (Vaishampayan et al., 1975; Coombe, 1982). However, it is unknown whether eggplant has a differentially-attractive visual spectrum compared to poinsettia. In addition to visual orientation, olfactory plant cues might facilitate rapid movement of whiteflies to eggplant. Vaishampayan et al. (1975) found that olfactory cues were necessary for *T. vaporariorum* to distinguish between host plants in conjunction with visual cues. Morphological and behavioral studies have shown that the antennae of *T. vaporariorum* contain olfactory receptors (Mellor & Anderson, 1995a,b). In addition to the fast attraction to eggplant, gustatory information from probing plant sap is thought to play a key role in arresting adult whiteflies on eggplant after landing on the plant (van Lenteren & Noldus, 1990).

When adult *T. vaporariorum* were allowed to first settle on poinsettia and then were provided with eggplant, the poinsettia-strain was less responsive to eggplant than

the bean-strain: 60% of the poinsettia-strain was attracted to eggplant during the 3-day observation period, whereas 91% of the bean-strain was found on eggplant. When the similar sequential choice was provided in the greenhouse for an extended time period (5 days), the poinsettia-strain showed a delayed attraction to eggplant, but the two whitefly strains showed an equivalent level of accumulation on eggplant at the end of the experiment. Therefore, our data suggest that the experience of *T. vaporariorum* on poinsettia resulted in delayed movement to eggplant but did not alter final accumulation on eggplant. It is likely that the experience on poinsettia caused adult whiteflies to feed longer on poinsettia, compared to the bean-strain, and therefore delayed movement to eggplant. Lei et al. (1998) demonstrated that adult *T. vaporariorum* probed longer and fed more persistently on the host plants for which the insects had experience.

Multi-generational rearing of *T. vaporariorum* on poinsettia significantly enhanced adult survivorship on poinsettia compared to whiteflies reared on bean. However, both whitefly strains showed a relatively low survivorship of adults on poinsettia; no more than 50% of the adults released onto poinsettia were observed on the plant after 3 days. Given that adult whiteflies are highly vulnerable to death due to starvation or dehydration when the insects are not on a host plant for even a short time period (<24 h) (Fenigstein et al., 2001), the observed rapid decrease in whitefly numbers on the plant is a good measure of whitefly mortality. In contrast, although neither whitefly strain had experience on eggplant, most adult whiteflies, that were attracted to eggplant, were strongly retained on the eggplant over 3 days. This indicates that poinsettia might not be a highly suitable host for *T. vaporariorum* even

though this whitefly species can be a major pest on poinsettia (Van Driesche et al., 1999, 2001; Murphy et al., 2007). Apparently, *T. vaporariorum* has a wide host range, but some plants such as sweet pepper (*Capsicum annum* L.) are acceptable for this whitefly species, though marginally suitable (van Lenteren & Noldus, 1990; Thomas, 1993a,b): the whiteflies did not substantially enhance their performance (e.g., survivorship) on the marginal host sweet pepper, even after multi-generation rearing. However, to our knowledge, there is no empirical assessment of relative host suitability of poinsettia for *T. vaporariorum* in a comparison with other host plants.

The observed pattern suggests that plant traits including chemical defense (Jiménez et al., 1995) or physical properties (Cohen et al., 1996) served as a key factor in determining the baseline suitability of a given host for *T. vaporariorum* with a slight tuning following host experience. Lei et al. (1998) found that host plants had a much stronger influence on the probing and feeding activities of *T. vaporariorum* than whitefly strains. A comparable pattern was also observed with the sweetpotato whitefly (*B. tabaci*) for their egg-laying pattern (Veenstra & Byrne, 1998). The authors found in non-choice experiments that *B. tabaci* deposited significantly more eggs on melon (*Cucumis melo* L.) than on cotton *Gossypium hirsutum* L., regardless of their experience on either plant. Again, this demonstrates that host plant type is a major factor that influences the performance of the whiteflies (e.g., fecundity) on a given plant.

In our previous study, we found that a high attractiveness of eggplant might be useful in a trap crop system if the survivorship of adult whiteflies on the cash crop is relatively high (Lee et al., 2009). This is because the effectiveness of trap cropping, as

reflected by actual reductions in the pest density on the cash crop, is determined not only by the attraction of whiteflies to the trap crop but also by their survivorship on the cash crop. The results of this study indicate that the poinsettia-strain had higher adult survivorship on poinsettia, compared to the bean-strain, and showed a similar level of preference to eggplant over poinsettia. This led us to predict that the use of eggplant as a trap crop would be more effective for the poinsettia-reared whiteflies. Indeed, the high attractiveness of eggplants consistently resulted in lower numbers of the poinsettia-strain adults and their progeny on poinsettias under the trap cropping, compared to poinsettias in monoculture. In addition to being highly attracted to eggplant, the whiteflies were also strongly arrested by the trap crop, thereby preventing the eggplants from becoming a source of the pests after attracting them. This was evidenced by there being no increase in the whitefly density on the poinsettias neighboring the eggplants, compared to the poinsettias at the same location in monoculture. In contrast, our previous study showed that there were hotspots of whitefly nymphs on poinsettias neighboring eggplants and these hotspots resulted in a higher nymph density under the trap cropping compared to that in monoculture (Lee et al., 2009). This is an apparent contradiction because our experiments have shown that adults from the bean-strain move more quickly to eggplant than those from the poinsettia-strain which should result in a pattern opposite to what we observed. It is likely that for both strains there is a probability for hotspots of nymphs to form around the eggplant trap crop but because this is a stochastic process, it was not evident in the experiments with the poinsettia-strain of whiteflies.

Although the trap cropping reduced whitefly density on the cash crop, it is

noteworthy that the difference in the number of adult *T. vaporariorum* on poinsettias between the two treatments was much smaller than the number of whiteflies that were attracted to eggplant. Actual reductions in the whitefly abundance on poinsettias by the trap crop after 24 h were equivalent only to 9-56% of the number of whiteflies observed on eggplants. This diluted effect of the trap cropping was most likely due to the relatively low survivorship of the poinsettia-strain adults on poinsettia. As a result, the use of eggplant as a trap crop was somewhat effective for the poinsettia-reared whiteflies, but the trap crop appeared to rescue a large number of adults from death that would have occurred had they remained on the poinsettias. The observed pattern highlights the need to distinguish between the apparent attractiveness of a trap crop and the actual effectiveness of the trap cropping. Given that anecdotal evidence for the potential of trap cropping is largely based on the accumulation of the pests on a trap crop and that this may not be indicative of actual effectiveness, a potential trap crop system must be evaluated in manipulative experiments to quantify actual reductions of pest density on the cash crop that result from deployment of the trap crop.

The traditional view of trap cropping can be expanded to situations where a trap crop attracts and kills pests (Shelton & Nault, 2004) or simply kills (Wu et al., 2008). In the first case the trap crop must be very attractive but there would not be accumulation of the pest on the trap crop. In the latter case the attractiveness criterion can be dropped because temporal availability of crop resources or pest mobility may put many of the target pests within the trap crop (e.g., a transgenic Bt crop). Even in these cases though there must be a demonstrable impact on pest density following deployment of the trap crop in order for the trap crop to be deemed effective. Even in

a trap and kill situation, we believe that the effectiveness of the trap crop will likely be low if the relative pest survivorship on the cash crop is low unless the intent is just to temporarily displace the pest as in a vector management program. Although the trap crop does not 'rescue' pests, as in our case, mortality of the pest is high on the cash crop and therefore, there is little potential for the trap crop to add to it. When a trap crop simply kills a target pest, the abundance on the cash crop must again be high enough for the mortality in the trap crop to make a difference in pest abundance on the cash crop.

In this study, we investigated the effectiveness of trap cropping after the pest population was reared for generations on to the cash crop. Our data indicate that experience of whiteflies on poinsettia enhanced their survivorship on poinsettia while maintaining their preference to eggplant over poinsettia. As expected, this change contributed to an increase in the effectiveness of the trap cropping. However, as adult whitefly survivorship on poinsettia was relatively low even after the pests were reared on the cash crop for several generations, a large proportion of adult whiteflies on the trap crop did not result in a reciprocal density reduction in the cash crop. Our findings emphasize that pest mortality on the cash crop strongly influences trap cropping effectiveness and the apparent accumulation of adult whiteflies on a trap crop should not be interpreted as a measure of trap cropping effectiveness.

REFERENCES

Al-Musa A (1982) Incidence, economic importance, and control of tomato yellow leaf curl in Jordan. Plant Disease 66: 561–563.

van Boxtel W (1980) Determination of Host-Plant Quality of Eggplant, Cucumber, Tomato and Sweet Pepper for *Trialeurodes vaporariorum* (in Dutch). MSc Thesis, Leiden University, Leiden, The Netherlands.

Byrne DN & Bellows TS (1991) Whitefly biology. Annual Review of Entomology 36: 431–457.

Castle SJ (2006) Concentration and management of *Bemisia tabaci* in cantaloupe as a trap crop for cotton. Crop Protection 25: 574–584.

Cohen AC, Henneberry TJ & Chu CC (1996) Geometric relationships between whitefly feeding behavior and vascular bundle arrangements. Entomologia Experimentalis et Applicata 78: 135–142.

Coombe PE (1982) Visual behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum*. Physiological Entomology 7: 243-251.

Dorsman R & van de Vrie M (1987) Population dynamics of the greenhouse whitefly *Trialeurodes vaporariorum* on different gerbera varieties. IOBC/WPRS Bulletin 10(2): 46–51.

Ellsworth PC, Chernicky JP, Byrne DN, Gibson R & Meade D (1992) A native weed as a trap crop for whiteflies in cotton. Proceedings Beltwide Cotton Insect Research and Control Conference (ed. by DJ Herber), pp. 911–913. National Cotton Council, Nashville, TN, USA.

Fenigstein A, Eliyahu M, Gan-Mo S & Veierov D (2001) Effects of five vegetable oils on the sweetpotato whitefly *Bemisia tabaci*. Phytoparasitica 29: 197–206.

Greenberg SM, Jones WA & Liu T-X (2002) Interactions among two species of *Eretmocerus* (Hymenoptera: Aphelinidae), two species of whiteflies (Homoptera: Aleyrodidae), and tomato. Environmental Entomology 31: 397–402.

Hilje L, Costa HS & Stansly PA (2001) Cultural practices for managing *Bemisia tabaci* and associated viral diseases. Crop Protection 20: 801–812.

Hokkanen HM (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119–138.

Jiménez DR, Yokomi RK, Mayer RT & Shapiro JP (1995) Cytology and physiology of silverleaf whitefly-induced squash silverleaf. Physiological and Molecular Plant Pathology 46: 227–242.

Lee D-H, Nyrop JP & Sanderson JP (2009) Attraction of *Trialeurodes vaporariorum* and *Bemisia argentifolii* to eggplant, and its potential as a trap crop for whitefly management on greenhouse poinsettia. Entomologia Experimentalis et Applicata 133: 105–116.

Lei H, Tjallingii WF & van Lenteren JC (1998) Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. Entomologia Experimentalis et Applicata 88: 73–80.

van Lenteren JC & de Ponti OMB (1990) Plant-leaf morphology, host plant resistance and biological control. Symposia Biologica Hungarica 39: 365–386.

van Lenteren JC & Noldus LPJJ (1990) Whitefly-plant relationships: behavioral and ecological aspects of whitefly-plant relationships. Whiteflies: Bionomics, Pest

Status and Management (ed. by D Gerling), pp. 47–89. Intercept, Andover, UK.

McAuslane HJ, Johnson FA, Colvin DL & Sojack B (1995) Influence of foliar pubescence on abundance and parasitism of *Bemisia argentifolii* on soybean and peanut. Environmental Entomology 24: 1135–1143.

Mellor HE & Anderson M (1995a) Antennal sensilla of whiteflies: *Trialeurodes vaporariorum* (Westwood), the glasshouse whitefly, *Aleyrodes proletella* (Linnaeus), the cabbage whitefly, and *Bemisia tabaci* (Gennadius), the tobacco whitefly (Homoptera: Aleyrodidae). Part 1: External morphology. International Journal of Insect Morphology and Embryology 24: 133–143.

Mellor HE & Anderson M (1995b) Antennal sensilla of whiteflies: *Trialeurodes vaporariorum* (Westwood), the glasshouse whitefly, and *Aleyrodes proletella* (Linnaeus), the cabbage whitefly, (Homoptera: Aleyrodidae). Part 2: Ultrastructure. International Journal of Insect Morphology and Embryology 24: 145–160.

Murphy GD, Short M, Cooper A-M, Fast M, Neal D & Jandricic S (2007) Biological control of whitefly in poinsettia in Ontario, Canada. Fourth International Bemisia Workshop International Whitefly Genomics Workshop (ed. by PA Stansly & CL McKenzie). Journal of Insect Science 8: 4, available online: insectscience.org/8.04.

Noldus LPJJ, Xu RM & van Lenteren JC (1986) The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera, Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera, Aleyrodidae) XIX. Feeding site selection by the greenhouse whitefly. Journal of Applied Entomology 101: 492–507.

Perring TM, Mayberry KS & Natwick ET (1995) Silverleaf whitefly management in cauliflower using a trap crop. Silverleaf Whitefly: 1995 Supplement to the 5-year National Research and Action Plan (ed. by TJ Henneberry, NC Toscano, RM Faust & JR Coppedge), p. 151. USDA-ARS, Washington, DC, USA.

Schuster DJ (2004) Squash as a trap crop to protect tomato from whitefly-vectored tomato yellow leaf curl. International Journal of Pest Management 50: 281–284.

Shelton AM & Badenes-Perez FR (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Shelton AM & Nault BA (2004) Dead-end trap cropping: a technique to improve management of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Crop Protection 23: 497–503.

Smith HA, Koenig RL, McAuslane HJ & McSorley R (2000) Effect of silver reflective mulch and a summer squash trap crop on densities of immature *Bemisia argentifolii* (Homoptera: Aleyrodidae) on organic bean. Journal of Economic Entomology 93: 726–731.

Smith HA & McSorley R (2000) Potential of field corn as a barrier crop and eggplant as a trap crop for management of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on common bean in north Florida. Florida Entomologist 83: 145–158.

Thomas DC (1993a) Host Plant Adaptation in the Glasshouse Whitefly. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Thomas DC (1993b) Host plant adaptation in the glasshouse whitefly. VI. Can a Dutch strain of whitefly adapt to a Dutch strain of sweet pepper? Journal of Applied Entomology 115: 405–415.

Vaishampayan SM, Waldbauer GP & Kogan M (1975) Visual and olfactory responses in orientation to plants by the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Entomologia Experimentalis et Applicata 18: 412–422.

Van Driesche RG, Hoddle MS, Lyon S & Sanderson JP (2001) Compatibility of insect growth regulators with *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control on poinsettia. Biological Control 20: 132–146.

Van Driesche RG, Lyon SM, Hoddle MS, Roy S & Sanderson JP (1999) Assessment of cost and performance of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control in commercial poinsettia crops. Florida Entomologist 82: 570–594.

Veenstra KH & Byrne DN (1998) The effects of physiological factors and host plant experience on the ovipositional activity of the sweet potato whitefly, *Bemisia tabaci*. Entomologia Experimentalis et Applicata 89: 15–23.

van Vianen A, Xu RM & van Lenteren JC (1988) The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). XXVII. Feeding site selection by the greenhouse whitefly on different host-plant species. Journal of Applied Entomology 105: 149–153.

Wu K-M, Lu Y-H, Feng H-Q, Jiang Y-Y & Zhao J-Z (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321: 1676–1678.

CHAPTER 4*

AVOIDANCE OF NATURAL ENEMIES BY ADULT WHITEFLIES, *BEMISIA ARGENTIFOLII*, AND EFFECTS ON HOST PLANT CHOICE

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Abstract

In this study we asked whether, in the context of a trap crop system, differential predation risks among plants influence host choice patterns of adult whiteflies, *Bemisia argentifolii*. We investigated whether adult whiteflies avoid natural enemies inhabiting poinsettia (a cash crop) and whether this behavior can be used to increase the movement of whiteflies to cucumber (a trap crop). The potential of cucumber as a trap crop was first evaluated and we found that significantly more whiteflies were attracted to cucumber when the whiteflies were released between the two plants. However, the accumulation of whiteflies on cucumber substantially diminished if the insects had first settled on poinsettia. Under such circumstances, we investigated whether movement of adult whiteflies to cucumber could be increased by creating conditions that would cause the whiteflies that had settled on poinsettia to leave the plant. A mechanical disturbance, consisting of shaking the plant, was first used to test this hypothesis. The shaking caused more whiteflies to leave poinsettia and move onto the trap crop, compared to undisturbed whiteflies. We then asked whether the presence of natural enemies on the cash crop could induce whiteflies to leave the cash crop and move onto the trap crop. Three natural enemies were tested: two predators, *Amblyseius*

swirskii and *Delphastus catalinae*, and a parasitoid *Encarsia formosa*. The presence of *D. catalinae* on poinsettia induced significantly more whiteflies to disperse to cucumber compared to poinsettia with no natural enemies, whereas *A. swirskii* and *E. formosa* did not result in a significant increase. Predator avoidance behavior by adult whiteflies should be investigated further in the context of trap cropping and other crop-habitat alterations designed to help manage whitefly abundance.

Introduction

The emphasis in arthropod biological control has traditionally been to make use of natural enemy consumption of prey in order to manage pest abundance. This focus has yielded many effective biological control programs but it has largely ignored an important aspect of the dynamics that unfold among insect pests and their arthropod natural enemies. Arthropods respond to risks from predation and parasitism by changing activity level and/or habitat use (Lima, 1998). These changes, collectively classified as non-consumptive effects of natural enemies, can substantially influence the abundance of herbivorous insects and the plant damage that these insects cause (Nelson et al., 2004; Nelson & Rosenheim, 2006; Thaler & Griffin, 2008). It is now apparent that non-consumptive effects by predators are important in many food webs and could even exceed the magnitude of the consumptive effects in regulating prey populations (Relyea, 2001; Werner & Peacor, 2003; Luttbeg & Kerby, 2005; Preisser et al., 2005; Thaler & Griffin, 2008). Important crop pests including whiteflies (Nomikou et al., 2003), aphids (Nelson, 2007), spider mites (Škaloudová et al., 2007) and thrips (Walzer & Schausberger, 2009) have been shown to alter feeding activity or

host choice in response to the presence of natural enemies. Despite the recognized importance of non-consumptive effects and that these effects are manifest by important crop pests, there are few overt considerations of these behaviors in pest management programs (Walzer & Schausberger, 2009). In this paper we report the results of experiments intended to determine whether natural enemies induce adult silverleaf whitefly, *Bemisia argentifolii* (Hemiptera: Aleyrodidae) to increase movement from one host plant to another. Consideration of this process was motivated by efforts to increase the effectiveness of trap cropping for this whitefly by determining whether natural enemies, when present on a cash crop, can "push" whiteflies towards a trap crop.

The silverleaf whitefly is a serious pest in many agricultural systems. This whitefly exhibits different mobility and vulnerability to predation over its life cycle; the immature stages are sessile on leaves and vulnerable to predation, whereas the adults are mobile and far less vulnerable to attack from most natural enemies. Because of the dichotomy of the pest ontogeny, most work on biological control has examined the direct predation or parasitism by natural enemies on sessile immature whiteflies (Hoddle et al., 1998; Gerling et al., 2001; Naranjo, 2001). In contrast, very few studies have addressed the impact of natural enemies on mobile whitefly adults that can escape from the natural enemies. Recent studies have shown that adult *B. argentifolii* can learn to avoid host plants harboring predatory mites (Nomikou et al., 2003; Meng et al., 2006). That is, female adults avoid allocating their reproductive potential at places where their offspring would be exposed to high predation risks (Ohsaki & Sato, 1994; Mappes & Kaitala, 1995; Ballabeni et al., 2001).

This induced behavioral change of adult *B. argentifolii*, mediated by differential predation risk among host plants, may have an influence on the outcome of habitat manipulation tactics such as trap cropping where the goal is to attract target pests to a trap crop and away from a cash crop. Trap cropping is often assumed to be highly-compatible with biological control and the two tactics have been jointly implemented (Khan & Pickett, 2004; Shelton & Badenes-Perez, 2006; Murphy et al., 2007). In such circumstances, along with the intrinsic attractiveness of a trap crop to adult whiteflies, the distribution of natural enemies among plants might also play a role in determining the overall effectiveness of the combined use of trap cropping and biological control. As a result, the differential predation risk between trap crop and cash crop may need to be considered when simultaneously using the two management tactics. For example, if a higher predation risk is maintained on a cash crop compared to a trap crop, the differential predation risk might supplement the intrinsic attractiveness of the trap crop to adult whiteflies, thereby facilitating more whiteflies to move to the trap crop. The attraction and accumulation of natural enemies on the trap crop might also have an adverse effect if the presence of natural enemies on the trap crop significantly dampens the attractiveness of the trap crop to whiteflies, or even compels adult whiteflies to move back to the cash crop.

Considerable effort has been devoted toward developing trap crop systems for *Bemisia* spp.; however the effectiveness of trap cropping remains equivocal (Hilje et al., 2001). Some authors found that trap cropping was effective at reducing whitefly density or whitefly-vectored viruses on cash crops (Al-Musa, 1982; Ellsworth et al., 1992; Schuster, 2004; Castle, 2006); others have concluded that trap cropping did not

consistently reduce whitefly density (McAuslane et al., 1995; Perring et al., 1995; Smith & McSorley, 2000; Smith et al., 2000; Lee et al., 2009). The low reliability of trap cropping for *Bemisia* whiteflies might be due to the biological traits of the insects coupled with constraints in how trap cropping can be implemented. *B. argentifolii* tend to readily settle on acceptable plants and do not respond to an alternative host plant once they have done so (Bird & Krüger, 2006; Lee et al., 2009). Therefore, a preferred host plant (e.g., a trap crop) in a choice experiment does not guarantee a consistent attraction of whiteflies toward that plant once whiteflies probe and settle on an acceptable host plant (e.g., a cash crop) (Lee et al., 2009). In addition, the area devoted for trap cropping is generally less than 10% of the total production and this percentage can be much lower for high-value crops such as greenhouse vegetables and ornamentals (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). For example, the area devoted for trap cropping in poinsettia greenhouses was less than 1% of the production area (Murphy et al., 2007).

These limitations might be mitigated by integrating a "push component" into the trap crop system to induce more adult whiteflies to leave the cash crop and move to the trap crop (Cook et al., 2007). Hence, we conducted a series of experiments to address whether a higher predation risk on a cash crop, compared to that on a trap crop, could serve as such a push component. We first examined the potential of cucumber [*Cucumis sativus* (Cucurbitaceae)] as a trap crop for *B. argentifolii* on greenhouse poinsettia [*Euphorbia pulcherrima* (Euphorbiaceae)]. *B. argentifolii* is a major pest on poinsettia and cucumber is one of the most preferred host plants for this whitefly species (Al-Musa, 1982). We then conducted an experiment to determine

whether mechanically disturbing adult whiteflies that had settled on a cash crop plant could cause more of those adults to leave the cash crop and subsequently move to a trap crop plant. Finally, we asked whether adult whiteflies increased movement to a trap crop plant when natural enemies of their offspring were present on a cash crop plant. We tested three commercially-available natural enemies, each with different foraging and feeding behaviors: a predatory mite [*Amblyseius swirskii* (Acari: Phytoseiidae)], a predatory beetle [*Delphastus catalinae* (Coleoptera: Coccinellidae)], and a parasitoid wasp [*Encarsia formosa* (Hymenoptera: Aphelinidae)].

Materials and methods

Insects

Bemisia argentifolii (= *B. tabaci* biotype B) originated from a stock culture reared on poinsettia that was initiated in 1989 at Cornell University. The colony was maintained on bean [*Phaseolus vulgaris* L. (Fabaceae)] in a walk-in growth chamber at 20-24 °C and a L14:D10 photoperiod for two years. Three natural enemies, *A. swirskii*, *D. catalinae*, and *E. formosa*, were purchased from a commercial supplier (IPM Laboratories Inc., Locke, NY, USA) and stored at 10 ^{o}C <5 days before use in the experiments. *Encarsia formosa* were allowed to emerge for 2-3 days at room temperatures and adults were aspirated into a 25 ml glass vial for use in the experiments. Plants were grown in 15 cm pots with Pro-Mix soil (Premier Horticulture Inc., Quakertown, PA, USA) and fertilized (N-P-K: 21-5-20) 5 days a week in greenhouses at 20-25 °C and L14:D10 photoperiod. The plants were not treated with pesticides.

Response of whiteflies to trap crop

A choice experiment was first conducted to measure the preference of adult whiteflies for cucumber versus poinsettia. This experiment is referred to as an unconditional choice test because the whiteflies had no prior experience with either host plant. Experimental arenas were created in BugDorm-3120 cages (60 × 60 × 60 cm; MegaView Science Education Services Co., Taichung, Taiwan) or BugDorm-4180 cages (93 × 48 × 48 cm). The experiment was carried out at 20-25 °C and a L14:D10 photoperiod in a research greenhouse. One potted cucumber (cv '*Marketmore 76*'; 4-5 weeks old from seed) and one potted poinsettia (cv '*Freedom Red*'; 8-10 weeks old from propagated cutting) were placed 25 cm apart from center to center in a cage. The location of the two plants in a cage was randomly assigned to the left or right side of the cage. One-hundred adult whiteflies (mixed age and sex) were collected from the whitefly colony in a 25 ml glass vial. The vial was capped with a plastic lid and placed between the two plants and whiteflies were released by opening the plastic lid. The number of adult whiteflies on the underside of the leaves was counted by carefully lifting and tilting the potted plant. The counting rarely induced whiteflies to fly off from the plant (personal observation). Observations were made at 6, 24, 48, and 72 hours after the release. The test was replicated 12 times with six replicates during each of two time periods.

The data were analyzed using repeated measures ANOVA. The response variable was expressed as the number of whiteflies on the cucumber minus the number on the poinsettia. Thus, the response variable represents the preference of whiteflies

for either plant in an experimental arena (i.e., cage) with a positive value indicating a preference for cucumber. A linear mixed model was used to test whether the response variable was greater than zero. In the model, the fixed factor was time and the random factors were date of replication, cage type, and cucumber location in cage. The model was tested for statistical significance using PROC MIXED in SAS (SAS Institute, Cary, NC, USA). The variation in repeated measurements was modeled using the covariance structure that produced the best model fit.

A second choice experiment was conducted to determine the extent to which adult whiteflies that had settled on poinsettia would move to cucumber. This experiment is referred to as a conditional choice test because the whiteflies had initially settled on poinsettia before they were provided a choice of another plant. Before the test, potted poinsettia plants (11 weeks old from propagated cuttings) were placed on a greenhouse bench (1.5 × 2.5 m) to infest the plants with adult whiteflies. One-hundred adults (mixed age and sex) were collected in a 25 ml glass vial and placed next to each poinsettia and the whiteflies released from the vial. After 24 hours, each whitefly-infested poinsettia was examined to record the number of adults settled on the plant. Each poinsettia was then carefully moved into a BugDorm cage containing one uninfested potted cucumber (4 weeks old from seed) (P+C) or one uninfested potted poinsettia (P+P). The two plants in a cage were placed 25 cm apart from center to center and their locations in a cage were randomly assigned to the left or right side. The number of adult whiteflies on the underside of the leaves was counted at 6, 24, 48, and 72 hours. The experiment was carried out at 20-25 °C and a L14:D10 photoperiod in a research greenhouse and the test was replicated 10 times.

The data were analyzed using repeated measures ANOVA. The response variable was expressed as the proportion of whiteflies on the uninfested plant (cucumber for P+C; poinsettia for P+P), relative to the total number of whiteflies on the plants in each cage. Thus, the response variable represents how many whiteflies moved from the poinsettia to the uninfested plant. The response variable was arcsine-transformed to normalize the data and equalize variances. In the ANOVA model, the fixed factors were time, plant combination, and their interaction; random factors were cage type and uninfested plant location in a cage. The model was tested for statistical significance using PROC MIXED in SAS.

Shaking disturbance

The objective of this experiment was to determine whether mechanical disturbance of adult whiteflies that had settled on poinsettia plants would cause more adults to move to cucumber plants. A preliminary experiment was first conducted to measure how many whiteflies left poinsettia after shaking the plant. Before the test, potted poinsettias (11 weeks old from propagated cutting) were placed individually into BugDorm cages and 100 adult whiteflies were released into the cage to infest the poinsettia. After 24 hours, the number of adults on the poinsettia was recorded. Then, the poinsettia was lifted 10 cm above the cage bottom and shaken ca. 25 times through a ca. 20 cm wide path; this took about 10 seconds. Immediately after shaking the plant, the number of adult whiteflies remaining on the plant was recorded. The test was replicated 8 times.

An experiment was then conducted to test whether shaking a plant to induce adult whiteflies to leave a poinsettia upon which they had settled would cause more adult whiteflies to move to a cucumber plant. Whitefly-infested potted poinsettias (11 weeks old from propagated cuttings) were prepared as described above and moved into a BugDorm cage containing one uninfested potted cucumber (5 weeks old from seed). The two plants in the cage were placed 35 cm apart from center to center. The shaking disturbance was applied to each poinsettia three times at 3-hour intervals; a control received no disturbance during the experiment. The number of adult whiteflies was recorded on the cucumber at 3, 6 and 24 hours. The experiments were carried out at 20-25 °C and a L14:D10 photoperiod in a research greenhouse. The test was replicated 5 times.

The data were analyzed using repeated measures ANOVA. The response variable was expressed as the proportion (percentage) of whiteflies on the cucumber at each observation time relative to the initial number of whiteflies on the poinsettia at the onset of the experiment. Thus, the response variable represents the cumulative movement of whiteflies from the poinsettia to the cucumber over time. The response variable was arcsine-transformed to normalize the data and equalize variances. In the ANOVA model, the main factors were time, treatment, and their interaction and were analyzed using Proc Mixed in SAS.

Natural enemy disturbance: cage trial

The objective of this experiment was to determine whether adult whiteflies avoid poinsettia plants that harbor certain natural enemies and whether this behavior can

increase the movement of whiteflies to a cucumber trap crop. Before the test, poinsettias (14 weeks old from propagated cuttings) were pruned to have a stem with ca. 9 leaves on each plant and placed individually into BugDorm cages. Two hundred adult whiteflies were released onto each poinsettia and allowed to oviposit so that the plant would be infested with immature whiteflies (eggs and nymphs). The immature whiteflies served as a food resource for the natural enemies and helped to retain natural enemies on the poinsettia. After 14 days, all adult whiteflies were removed from the poinsettia and then infested again with 100 adult whiteflies for use in the experiment. After 24 hours, each whitefly-infested poinsettia (with immatures and adults) was examined to record the number of adults and carefully moved into a BugDorm cage having one uninfested cucumber (7 weeks old from seed) inside. The two plants in a cage were placed 35 cm apart from center to center and their location was randomized.

Three natural enemies, the mite *A. swirskii*, the beetle *D. catalinae*, and the parasitoid *E. formosa*, were tested and were applied to the poinsettias in the cages as follows. For *A. swirskii*, ca. 10 adults were transferred by use of a fine paintbrush to each of five poinsettia leaf-discs (3 cm diameter) that were cut from 2-3 month old poinsettias. The five leaf-discs with predators were placed on the five upper leaves of each poinsettia. For *D. catalinae*, 3 adults were transferred using a paintbrush to each of five poinsettia leaf-discs which were then placed on the five upper leaves of each poinsettia. For *E. formosa*, 60 adults were aspirated into a 25 ml glass vial and released from the bottom of each poinsettia. Controls received no natural enemies. To provide an equal handling disturbance while introducing the natural enemies on the

poinsettia, either one sham vial or five sham leaf-discs with no natural enemies were placed in similar location across the treatments. The numbers of adult whiteflies on the underside of the leaves of each plant were counted every 24 hours for 3 days. The numbers of *D. catalinae* and *E. formosa* were counted simultaneously with whiteflies and if the natural enemies were found on the cucumber they were removed from the plant. The number of *A. swirskii* was recorded under a microscope after the 3-day observation. The experiment was carried out at 20-25 °C and a L14:D10 photoperiod in a research greenhouse. The test was replicated 5 times.

The data were analyzed using repeated measures ANOVA. The response variable was the proportion of whiteflies on the cucumber, relative to total number of whiteflies on the plants in each cage. The response variable was arcsine-transformed to normalize the data and equalize variances. In the ANOVA model, the fixed factors were time, treatment, and their interaction; the random factor was a spatial block (distance from a greenhouse chiller) that was used to minimize the possible effect of a gradient in the greenhouse environment. The linear mixed model was analyzed using Proc MIXED in SAS and the mean proportions were compared between each natural enemy treatment and control using the DUNNETT option. Significance values were adjusted for the multiple comparisons.

Natural enemy disturbance: greenhouse trial

Whether adult whiteflies avoid natural enemies and thereby increase movement to a natural enemy-free trap crop was evaluated at a large spatial scale. Each experimental arena consisted of one potted cucumber trap plant surrounded by six potted poinsettias

on a greenhouse bench. The six poinsettias (11-18 weeks old from propagated cuttings) were infested with immature whiteflies and placed 40 cm apart from center to center in a hexagonal configuration on a greenhouse bench (1.5 × 2.5m). Replicate greenhouse benches were separated floor-to-ceiling by curtains of Agribon+ AG-19 screen (Green Mountain Transplants, Arundel, ME, USA).

In contrast to the cage experiment in section 2.4, natural enemies were first applied to poinsettia plants and adult whiteflies were then released from the bottom of the poinsettias at the onset of the experiment. In the cage experiment, adult whiteflies were first allowed to settle on the plant and then natural enemies were introduced on the plant. As a result, there was a difference between the two experiments in the sequence and method of introducing adult whiteflies onto the poinsettia plants. In the current experiment, most adult whiteflies that were released at the bottom of the six poinsettias first settled on the poinsettia plants (see the Results). Therefore, this experiment provided the same situation as the cage trial where the adult whiteflies had settled on the poinsettia and the assessment was whether the natural enemies could induce more whiteflies to leave the poinsettia plants and accumulate on the cucumber trap plant.

For *A. swirskii*, ca. 20 adults were placed on each of five poinsettia leaf-discs (3 cm diameter) and transferred to the five upper leaves of each poinsettia. For *D. catalinae*, 30 adults were gently sprinkled on the upper leaves of each poinsettia. For *E. formosa*, 90 adults were aspirated into 25 ml glass vials and released from the bottom of each poinsettia. For the control plants, no natural enemies were applied. After applying the natural enemies, an uninfested cucumber (6 weeks old from seed)

was placed in the middle of the six poinsettias. Then, 50 adult whiteflies were released from the bottom of each poinsettia. The numbers of adult whiteflies and natural enemies on the plants were counted every 24 hours for 5 days. The numbers of *D. catalinae* and *E. formosa* were counted simultaneously with whiteflies and if the natural enemies were found on the cucumber they were removed from the plant. The number of *A. swirskii* was recorded under a microscope after the 5-day observation. The experiment was carried out at 20-25 °C and a L14:D10 photoperiod in a research greenhouse. Each natural enemy species was tested at a separate time with its own control. The test was replicated 4 times during two time periods, each with two replicates.

The data were analyzed using repeated measures ANOVA. The response variable was expressed as the proportion of whiteflies on the cucumber, relative to the total number of whiteflies on the plants in each experimental arena. The response variable was arcsine-transformed to normalize the data and equalize variances. In the ANOVA model, the fixed factors were time, treatment, and their interaction; the random factors were date of replication and greenhouse compartment. The linear mixed model was analyzed using Proc MIXED in SAS. In addition to the repeated ANOVA, the change over the duration of the experiment in the proportion of adult whiteflies on the cucumber was directly calculated within each replicate as the proportion at day 5 (P_5) minus that at day 1 (P_1). This difference ($P_\Delta = P_5 - P_1$) measures whitefly accumulation on the cucumber after adjusting for initial movement to the cucumber plant. The differences were compared between the natural enemy treatment and control using a one-sided t-test.

Results

Response of whiteflies to trap crop

In the unconditional choice setting where adult whiteflies were given a choice between poinsettia and cucumber, whiteflies settled on cucumber in significantly higher numbers than on poinsettia ($t = 3.02$, d.f. = 11, $P = 0.0117$) (Figure 4.1A). Six hours after releasing the whiteflies, 77% of the adult whiteflies were observed on cucumber and this proportion did not change over time ($F_{3,33} = 2.83$, $P = 0.0533$). In the conditional choice experiment where adult whiteflies were first allowed to settle on poinsettia, significantly more adult whiteflies remained on the poinsettia throughout the study, regardless of the uninfested plant species that was provided next to the poinsettia (P+C: $t = 8.59$, d.f. = 18, $P < 0.0001$; P+P: $t = 5.78$, d.f. = 18, $P < 0.0001$) (Figure 4.1B). The proportions of adult whiteflies on the uninfested plants increased over time in both plant combinations ($F_{3,54} = 19.57$, $P < 0.0001$); however, the proportion did not exceed 30% in any observation. Adults did not show a higher level of movement to the uninfested cucumber (P+C) compared to movement to the uninfested poinsettia (P+P) ($F_{1,18} = 0.27$, $P = 0.6125$).

Shaking disturbance

Immediately after shaking a poinsettia infested with adult whiteflies, 53% (range of 39–66%) of the whiteflies, having observed on the plant before the shaking, were observed on the plant. This indicates that 47% of the whiteflies on average moved off from the leaves and were in flight (data not shown). In the choice setting in which an

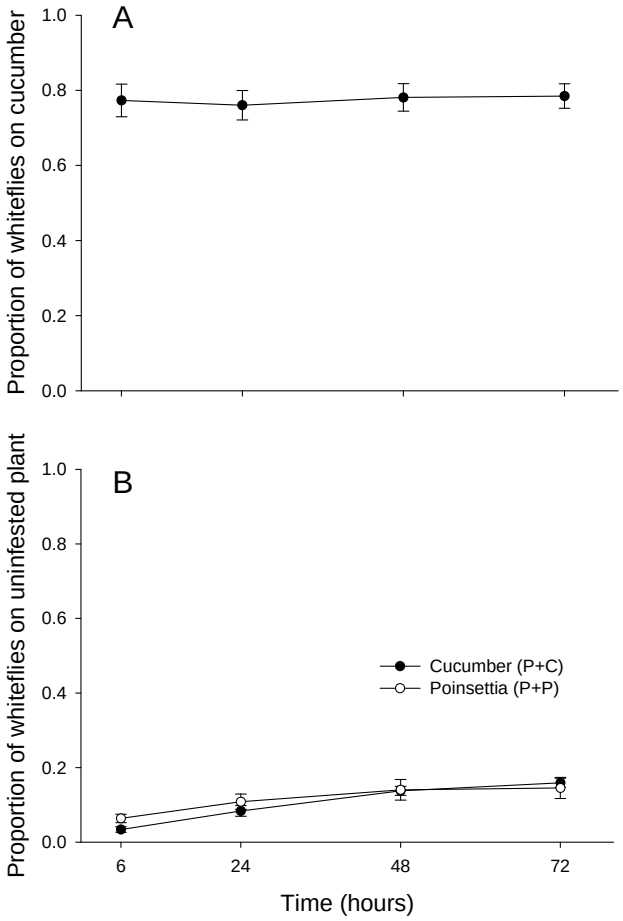

Figure 4.1. (A) Mean proportion (± SEM) of adult whiteflies observed on cucumber over time in an unconditional choice experiment. Whiteflies were released midway between a potted poinsettia and potted cucumber that had been placed in cage. (B) Mean proportion (± SEM) of adult whiteflies observed on an uninfested plant over time in a conditional choice experiment. Whiteflies had settled on the poinsettia and were then provided with an uninfested cucumber (P+C) or an uninfested poinsettia (P+P).

uninfested cucumber was provided next to a poinsettia, applying the shaking disturbance to the poinsettia resulted in a faster accumulation of adult whiteflies on the cucumber over time, compared to that in the control ($F_{2,16} = 8.10$, P = 0.0037) (Figure 4.2). As a result, 37% of the whiteflies moved from the poinsettia to the cucumber during 24 hours, whereas 13% of the whiteflies did so under no disturbance ($F_{1,8} = 8.64$, P = 0.0187).

Natural enemy disturbance: cage trial

Adult whiteflies showed different levels of movement to the cucumber among the treatments over time ($F_{6,32} = 3.06$, P = 0.0176) (Figure 4.3). The proportions of adult whiteflies on cucumber were not significantly different between any of the natural enemy treatments and controls during the first two days of the trial (*A. swirskii*: t = 1.28, d.f. = 12, P = 0.4614; *D. catalinae*: t = 1.54, d.f. = 12, P = 0.3278; *E. formosa*: t = 0.04, d.f. = 12, P = 0.9999). However, on the third day significantly more adult whiteflies in the *D. catalinae* treatment had moved from the poinsettia to the cucumber compared to the control (t = 2.85, d.f. = 12, P = 0.0374). The proportions of adult whiteflies on the cucumber at day three were not significantly greater in the other two treatments compared to controls (*A. swirskii*: t = 2.10, d.f. = 12, P = 0.1378; *E. formosa*: t = 2.11, d.f. = 12, P = 0.1337). Most of the natural enemies were found on the poinsettia during the study, indicating that higher predation risks were created and maintained on the poinsettia relative to the cucumber (Table 4.1).

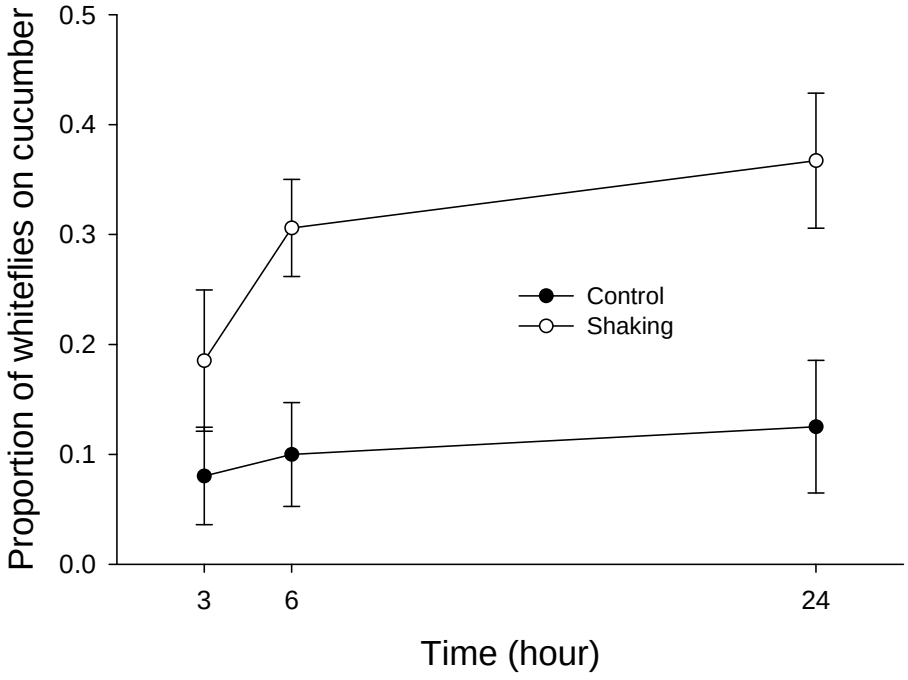

Figure 4.2. Mean proportion (± SEM) of adult whiteflies observed on cucumber over time in the shaking disturbance experiment. A potted poinsettia was initially infested with adult whiteflies and placed next to a potted cucumber in a cage. The shaking disturbance was given to the poinsettia at 0.5, 3.5, and 6.5 hours; controls received no disturbance.

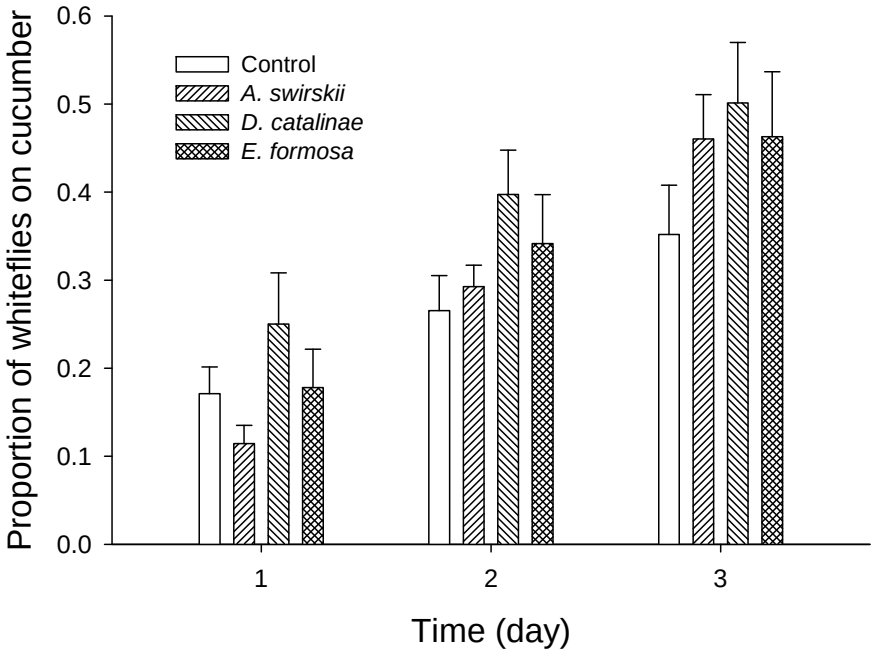

Figure 4.3. Mean proportion (± SEM) of adult whiteflies observed on cucumber over time in the natural enemy disturbance experiment in a cage. A potted poinsettia was initially infested with immature and adult whiteflies and placed next to a potted cucumber in a cage. Fifty *A. swirskii*, 15 *D. catalinae*, or 60 *E. formosa* were applied to the poinsettia; controls received no natural enemy.

Table 4.1. Mean number ± SEM of natural enemies recaptured on plants in the cage and greenhouse experiments

Experiment	Time (day)	Treatment					
		A. swirskii[b]		*D. catalinae*[c]		*E. formosa*[c]	
		Poinsettia	Cucumber	Poinsettia	Cucumber	Poinsettia	Cucumber
Cage (N = 5)	0[a]	50.00	0.00	15.00	0.00	60.00	0.00
	1	n/a	n/a	5.60 ± 1.29	0.00	15.20 ± 2.24	0.20 ± 0.20
	2	n/a	n/a	5.60 ± 0.40	0.00	14.00 ± 1.30	0.60 ± 0.40
	3	7.20 ± 2.03	0.60 ± 0.40	4.00 ± 0.89	0.00	10.20 ± 1.11	0.00
Greenhouse (N = 4)	0[a]	100.00	0.00	30.00	0.00	90.00	0.00
	1	n/a	n/a	9.21 ± 0.93	0.00	39.67 ± 7.21	5.75 ± 1.25
	3	n/a	n/a	3.96 ± 0.30	1.25 ± 0.95	35.42 ± 11.89	6.50 ± 1.76
	5	21.5 ± 2.78	0.00	2.88 ± 0.65	0.00	22.33 ± 8.90	7.00 ± 1.73

[a]The initial release number of each natural enemy per plant. [b]The number of *A. swirskii* was recorded under the microscope at the end of the experiments. [c]The numbers of *D. catalinae* and *E. formosa* were recorded while counting whiteflies; When the natural enemies were found on cucumber during the counting, they were removed from the plant.

Natural enemy disturbance: greenhouse trial

When a larger experimental arena was used, a similar pattern was observed in the movement of adult whiteflies from poinsettia to cucumber as in the cage trial. For *D. catalinae*, significantly more adult whiteflies were observed on the cucumber in the natural enemy treatment compared to the control ($F_{1,6} = 23.30$, P = 0.0029) (Figure 4.4A). Although the difference in the proportions between the two treatments consistently increased over time, the interaction term between time and treatment was not significant ($F_{4,24} = 0.63$, P = 0.6436). However, when the increase (P_{Δ}) in the proportions of whiteflies on the cucumber was calculated between day 1 and 5 within each replicate and directly compared between the natural enemy treatment and control, the increase in the proportions was significantly greater in the natural enemy treatment (t = 3.23, d.f. = 5, P = 0.0116). For *A. swirskii*, there was no significant difference in the proportion of adult whiteflies on the cucumber between the natural enemy and control treatments ($F_{1,6} = 1.22$, P = 0.3116) although the trends in the data are suggestive of a natural enemy effect (Figure 4.4B). There was no significant interaction between time and treatment ($F_{4,24} = 1.13$, P = 0.3653) and P_{Δ} was not significantly greater in the natural enemy treatment (t = 1.22, d.f. = 5, P = 0.1388). For *E. formosa*, there was no significant difference in the proportion of adult whiteflies on the cucumber between the two treatments ($F_{1,6} = 0.21$, P = 0.6626) nor was there a significant interaction between time and treatment ($F_{4,24} = 0.34$, P = 0.8509) (Figure 4.4C). Likewise, P_{Δ} was not significantly greater in the natural enemy treatment (t = 0.87, d.f. = 5, P = 0.2124). Most of *A. swirskii* and *D. catalinae* were observed on the

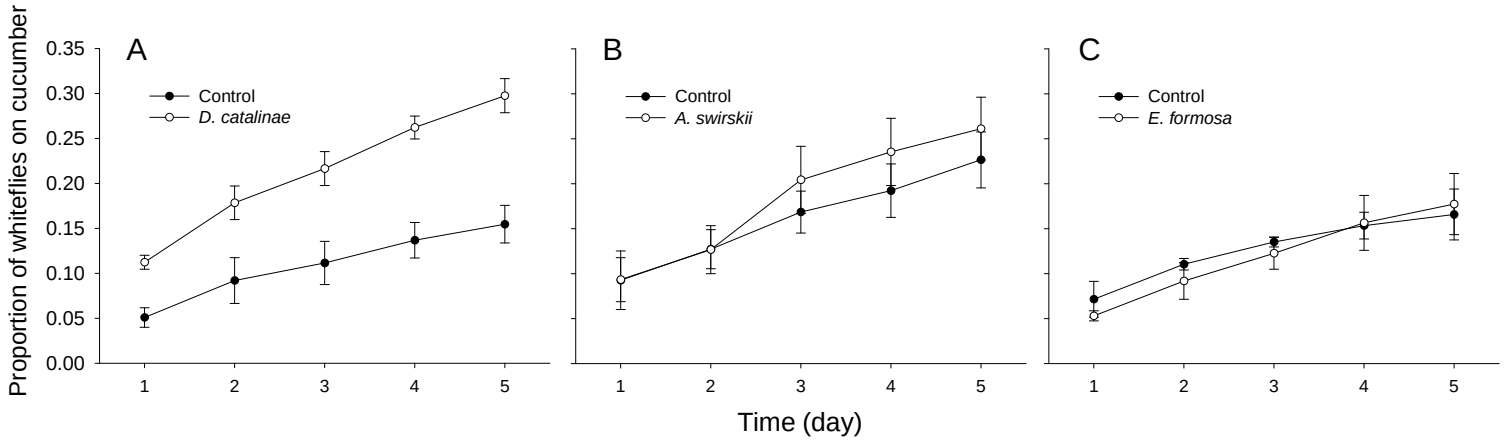

Figure 4.4. Mean proportion (± SEM) of adult whiteflies observed on cucumber over time in the natural enemy disturbance experiment on a greenhouse bench. Six poinsettias were initially infested with immature whiteflies and natural enemies and placed in a hexagon configuration. One-hundred *A. swirskii*, 30 *D. catalinae*, or 90 *E. formosa* were applied per poinsettia; controls receive no natural enemy. One cucumber was placed in the middle of the hexagon. Adult whiteflies were released at the bottom of each poinsettia plant.

poinsettia during the study, whereas *E. formosa* consistently dispersed into the cucumber (Table 4.1).

Discussion

When adult *B. argentifolii* were provided an initial choice between cucumber and poinsettia, the adults significantly preferred cucumber over poinsettia. However, cucumber did not effectively draw adult whiteflies from poinsettia if the adults had first settled on the poinsettia; less than 20% of adult whiteflies moved from the poinsettia to the cucumber over 3 days even when the two plants were in close proximity in the cage. In a previous study by Lee et al. (2009), eggplant failed to attract a sufficiently large number of adult *B. argentifolii* from poinsettia to function as a trap crop, although whiteflies preferred eggplant over poinsettia when initially given a choice between the two plants. This altered response of the whiteflies to potential trap crops, after having settled on the cash crop, is a challenge to successful trap cropping in poinsettia greenhouse systems. Our results indicate that adult *B. argentifolii* readily accept a poinsettia cash crop upon landing and do not further respond to a more-preferred trap crop. This pattern is consistent with results of behavioral observations on adult *Bemisia* whiteflies. For example, Bird & Krüger (2006) found that *B. tabaci* females remained mostly stationary on the same plant and movement among different hosts were rare in their continuous recordings.

Disruption of *Bemisia* whiteflies that have settled on a cash crop is needed to improve trap crop efficacy. This is because the majority of adult *B. argentifolii* that are

airborne choose to settle on cucumber over poinsettia; pushing whiteflies off a poinsettia cash crop might facilitate greater whitefly movement toward and accumulation on a cucumber trap crop. This is supported by the shaking experiment that we conducted. Given that adult whiteflies are highly vulnerable to death due to starvation or dehydration if the insects are forced to be away from plants for even a short time period (e.g., <24 hours) (Fenigstein et al., 2001), disturbing the whiteflies may also help increase direct mortality of the adults.

When natural enemies were tested for their potential as a push component, adult *B. argentifolii* responded differently to the three natural enemies tested. The presence of *D. catalinae* on poinsettia caused significantly more adult whiteflies to disperse to cucumber, compared to a control with no predators. In contrast, the presence of *A. swirskii* or *E. formosa* on the poinsettia did not result in a significant increase in the movement of adult whiteflies to the cucumber. In most cases, *D. catalinae* were observed only on the poinsettia on which immature whiteflies were provided as food; however, the number of *D. catalinae* on the poinsettia decreased rapidly in both cage and greenhouse trials. Thus, a higher predation risk was created on poinsettia compared to cucumber although this differential waned rather quickly. This may explain why the effect of *D. catalinae* on the distribution of adult *B. argentifolii* between the two plant species was greatest early in each experiment.

Given that *D. catalinae* is far more voracious than the other two species, it is possible that more immature whiteflies were fed upon by *D. catalinae*, especially during the first 24 hours of the study. Although we did not investigate how many immature whiteflies were consumed by each natural enemy species, the whitefly

conspecifics damaged by *D. catalinae* might have played a role in producing the observed pattern. A study with *Bemisia* whiteflies showed that the adult whiteflies avoided plants with predatory mites feeding on immature whiteflies but did not avoid plants with predators feeding on pollen (Meng et al., 2006), suggesting that the signal to which adult whiteflies responded was based in part on immature conspecifics that had been fed upon. In an aphid-parasitoid system, congeners that had been killed by parasitoids induced changes in aphid resource use and ultimately, led to a strong decline in aphid population size (Fievet et al., 2008). Therefore, at least some prey species appear to use cues from their predator-damaged congeners. In addition, of the three natural enemies tested, only *D. catalinae* poses a direct predation risk to adult whiteflies, though these predators are greatly impeded in attacking adult whiteflies when there are leaf trichomes and thus cannot feed on adult whiteflies (Guershon & Gerling, 1999). These structures are abundant on poinsettia.

It is noteworthy that the whitefly parasitoid *E. formosa* continuously moved from the poinsettia to the cucumber, which would likely dilute a differential predation risk between the cash crop and trap crop plants. This may explain why no differences in the distribution of adult whiteflies could be attributed to the presence of *E. formosa.* Under field conditions it would likely be impossible to consistently keep a low predation risk from *E. formosa* on a trap crop, compared to a cash crop.

In contrast to the parasitoid *E. formosa*, the predatory mite *A. swirskii* did not disperse from the poinsettia to the cucumber, most likely due to their limited mobility (Buitenhuis et al., 2010). However, despite the difference in abundance of *A. swirskii* between the poinsettia and cucumber, we did not observe a differential response by

adult *Bemisia* to the altered predation risk. This is in contrast to a previous study by Nomikou et al. (2003) who demonstrated that initial host choice by adult *Bemisia* whiteflies among cucumbers was significantly influenced by the presence of these predatory mites; adult whiteflies settled on plants with no predatory mites in significantly higher numbers compared to plants harboring the predators. However, in our experiments where adult whiteflies were first allowed to settle on poinsettia or released beneath the poinsettia, the presence of *A. swirskii* on poinsettia did not induce significantly more whiteflies to leave the poinsettia and move to a predator-free cucumber. The two studies suggest that, although adult whiteflies can detect and avoid plants harboring *A. swirskii* during the early stage of the host choice process, whiteflies might diminish avoidance behaviors once the adults have settled on an acceptable host plant.

Conclusions

In biological control, the consumptive effects on prey have been a key parameter in evaluating the potential of biological control agents. For decades, these efforts have produced tangible progress and outcomes such as commercialization of natural enemies and growing adoption of biological control programs by growers. However, a recent meta-analysis across diverse ecosystems indicates that non-consumptive predator effects can comprise more than 50% of the total predator effect on prey (Preisser et al., 2005). In addition, accumulating studies show that important herbivorous pests, including whiteflies, aphids, spider mites, and thrips, can alter their activity level and habitat use in response to the presence of predators. The current

study asked whether adult *Bemisia* whiteflies would change their plant choice pattern depending on the presence of natural enemies of their offspring among host plants, especially under a trap crop system. The results indicate that the predatory beetle, *D. catalinae* induced a significant change in the host choice pattern of the whiteflies and this behavioral change was substantial enough to augment the intrinsic attractiveness of a potential trap crop. That is, the results suggest that the avoidance behavior of the whiteflies might be utilized as a push component in trap cropping. However, it is also important not to dismiss possible negative impacts of the avoidance behavior on management outcomes. For instance, the application of certain predators or parasitoids may increase pest inflow into new, healthy host plants from an area of high pest density where natural enemies have been introduced or have moved to. It is also possible that application or dispersal of *D. catalinae* onto a trap crop can dilute the intrinsic attractiveness of the trap crop to whiteflies. Therefore, additional effort should be made to assess and better incorporate non-consumptive effects of natural enemies when designing and implementing bio-based management tactics.

REFERENCES

Al-Musa A (1982) Incidence, economic importance, and control of tomato yellow leaf curl in Jordan. Plant Disease 66: 561–563.

Ballabeni P, Wlodarczyk M & Rahier M (2001) Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? Functional Ecology 15: 318–324.

Bird TL & Krüger K (2006) Response of the polyphagous whitefly *Bemisia tabaci* B-biotype (Hemiptera: Aleyrodidae) to crop diversification – influence of multiple sensory stimuli on activity and fecundity. Bulletin of Entomological Research 96: 15–23.

Buitenhuis R, Shipp L & Scott-Dupree C (2010) Dispersal of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) on potted greenhouse chrysanthemum. Biological Control 52: 110–114.

Castle SJ (2006) Concentration and management of *Bemisia tabaci* in cantaloupe as a trap crop for cotton. Crop Protection 25: 574–584.

Cook SM, Khan ZR & Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annual Review of Entomology 52: 375–400.

Ellsworth PC, Chernicky JP, Byrne DN, Gibson R & Meade D (1992) A native weed as a trap crop for whiteflies in cotton. In: Herber, D.J. (Ed.), Proceedings Beltwide Cotton Insect Research and Control Conference. National Cotton Council, Nashville, TN, pp. 911–913.

Fenigstein A, Eliyahu M, Gan-Mor S & Veierov D (2001) Effects of five vegetable

oils on the sweetpotato whitefly *Bemisia tabaci*. Phytoparasitica 29: 197–206.

Fievet V, Lhomme P & Outreman Y (2008) Predation risk cues associated with killed conspecifics affect the behavior and reproduction of prey animals. Oikos 117: 1380–1385.

Gerling D, Alomar O & Arnó J (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Protection 20: 779–799.

Guershon M & Gerling D (1999) Predatory behavior of *Delphastus pusillus* in relation to the phenotypic plasticity of *Bemisia tabaci* nymphs. Entomologia Experimentalis et Applicata 92: 239–248.

Hilje L, Costa HS & Stansly PA (2001) Cultural practices for managing *Bemisia tabaci* and associated viral diseases. Crop Protection 20: 801–812.

Hoddle MS, Van Driesche RG & Sanderson JP (1998) Biology and use of the whitefly parasitoid *Encarsia formosa*. Annual Review of Entomology 43: 645–669.

Hokkanen HM (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119–138.

Khan ZR & Pickett JA (2004) The 'push-pull' strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. In: Gurr, G.M., Wratten, S.D., Altieri, M.A. (Eds.), Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods. CSIRO Publishing, Melbourne, Australia, pp. 155–164.

Lee D-H, Nyrop JP & Sanderson JP (2009) Attraction of *Trialeurodes vaporariorum* and *Bemisia argentifolii* to eggplant, and its potential as a trap crop for whitefly

management on greenhouse poinsettia. Entomologia Experimentalis et Applicata 133: 105–116.

Lima SL (1998) Nonlethal effects in the ecology of predator-prey interactions. BioScience 48: 25–34.

Luttbeg B & Kerby JL (2005) Are scared prey as good as dead? Trends in Ecology and Evolution 20: 416–418.

Mappes J & Kaitala A (1995) Host-plant selection and predation risk for offspring of the parent bug. Ecology 76: 2668–2670.

McAuslane HJ, Johnson FA, Colvin DL & Sojack B (1995) Influence of foliar pubescence on abundance and parasitism of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on soybean and peanut. Environmental Entomology 24: 1135–1143.

Meng R-X, Janssen A, Nomikou M, Zhang Q-W & Sabelis MW (2006) Previous and present diets of mite predators affect antipredator behaviour of whitefly prey. Experimental and Applied Acarology 38: 113–124.

Murphy GD, Short M, Cooper A-M, Fast M, Neal D & Jandricic S (2007) Biological control of whitefly in poinsettia in Ontario, Canada. In: Stansly, P.A., McKenzie, C.L. (Eds.), *Fourth International Bemisia Workshop International Whitefly Genomics Workshop*. Journal of Insect Science **8**, 4. http://insectscience.org/8.04.

Naranjo SE (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop Protection 20: 835–852.

Nelson EH (2007) Predator avoidance behavior in the pea aphid: costs, frequency, and population consequences. Oecologia 151: 22–32.

Nelson EH, Matthews CE & Rosenheim JA (2004) Predators reduce prey population

growth by inducing changes in prey behavior. Ecology 85: 1853–1858.

Nelson EH & Rosenheim JA (2006) Encounters between aphids and their predators: the relative frequencies of disturbance and consumption. Entomologia Experimentalis et Applicata 118: 211–219.

Nomikou M, Janssen A & Sabelis MW (2003) Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. Oecologia 136: 484–488.

Ohsaki N & Sato Y (1994) Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. Ecology 75: 59–68.

Perring TM, Mayberry KS & Natwick ET (1995) Silverleaf whitefly management in cauliflower using a trap crop. In: Henneberry, T.J., Toscano, N.C., Faust, R.M., Coppedge, J.R. (Eds.), Silverleaf Whitefly: 1995 Supplement to the 5-year National Research and Action Plan. USDA-ARS, Washington, DC, p. 151.

Preisser EL, Bolnick, DI & Benard MF (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86: 501–509.

Relyea RA (2001) The relationship between predation risk and antipredator responses in larval anurans. Ecology 82: 541–554.

Schuster DJ (2004) Squash as a trap crop to protect tomato from whitefly-vectored tomato yellow leaf curl. International Journal of Pest Management 50: 281–284.

Shelton AM & Badenes-Perez FR (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Škaloudová B, Zemek R & Křivan V (2007) The effect of predation risk on an acarine system. Animal Behaviour 74: 813–821.

Smith HA, Koenig RL, McAuslane HJ & McSorley R (2000) Effect of silver reflective mulch and a summer squash trap crop on densities of immature *Bemisia argentifolii* (Homoptera: Aleyrodidae) on organic bean. Journal of Economic Entomology 93: 726–731.

Smith HA & McSorley R (2000) Potential of field corn as a barrier crop and eggplant as a trap crop for management of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on common bean in north Florida. Florida Entomologist 83: 145–158.

Thaler JS & Griffin CAM (2008) Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. Entomologia Experimentalis et Applicata 128: 34–40.

Walzer A & Schausberger P (2009) Non-consumptive effects of predatory mites on thrips and its host plant. Oikos 118: 934–940.

Werner EE & Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.

CHAPTER 5

NON-CONSUMPTIVE EFFECTS OF PREDATOR, *DELPHASTUS CATALINAE*, ON HABITAT USE PATTERNS OF ADULT WHITEFLIES, *BEMISIA ARGENTIFOLII*,

Abstract

This study examined habitat choice patterns by adult whiteflies [*Bemisia argentifolii* (Hemiptera: Aleyrodidae)] in response to predators [*Delphastus catalinae* (Coleoptera: Coccinellidae)] at different spatial scales. When female whiteflies were confined in small arenas with leaf-discs from which they could not escape, most adult whiteflies readily settled on leaf-discs with no predators; however, whiteflies significantly delayed settling on leaf-discs when predators were present. The presence of *D. catalinae* altered the vertical distribution of adult whiteflies on cucumber plants; adult whiteflies moved upward faster over time within the plant canopy when predators were present compared to whiteflies on plants with no predators. Most *D. catalinae* were present on the lower part of the plants during the study. Therefore, we inferred that adult whiteflies more quickly moved to the upper plant strata to reduce the risk of predation of their progeny. Introduction of *D. catalinae* onto a cucumber plant with high whitefly density did not cause increased dispersal of adult whiteflies into neighboring uninfested plants. The results of this study indicate that predator-avoidance behaviors by adult *B. argentifolii* were different across different spatial scales. The predator-avoidance behavior may have a negative implication at the

within-plant scale by inducing more whiteflies to move into upper plant strata. However, the predator effect was not significant at the among-plant scale.

Introduction

Plants can be protected against damage from herbivorous arthropods when predators feed on the herbivores attacking the plants. This trophic interaction (i.e., the consumptive effects of predators) has been widely and intensively explored in biological control studies with the goal of developing pest management programs (Heinz et al., 2004). Indeed, there are many tangible outcomes such as commercial production of natural enemies and growing adoption of biological control by growers.

In predator-prey interactions, predators can influence prey population dynamics by inducing behavioral changes in prey as well as by feeding on prey (Lima, 1998; Relyea, 2001; Werner & Peacor, 2003; Luttbeg & Kerby, 2005; Thaler & Griffin, 2008). Accumulating evidence shows that prey may change habitat use and activity levels in response to predation risks; a recent meta-analysis across diverse ecosystems indicates that these changes, collectively classified as non-consumptive effects of predators, can compromise more than 50% of the total predator effect on prey (Preisser et al., 2005). Unlike direct predation, non-consumptive effects of predators may not result in an immediate reduction of target pest numbers. However, the non-lethal interactions can force prey to adopt defensive strategies and these anti-predator defenses can exert a suite of demographic effects. In terrestrial systems, it has been shown that the risk of predation leads to reduced foraging by prey which in turn can translate into reduced fecundity (Preisser & Bolnick, 2008).

The primary objective of biological control has been to reduce crop damage by herbivorous insects through the top-down forces of natural enemies. Most biological control studies assume that these top-down forces depend solely on consumptive pathways in predator-prey interactions. However, given that predators can induce a substantial decrease in foraging efficiency and effort by prey (Preisser & Bolnick, 2008), the cascading impacts of non-consumptive pathways on plant resource use by herbivores should be considered in the context of biological control. Recently, more efforts have been made to explicitly examine non-consumptive predator effects on crop pests in a pest management context (Nomikou et al., 2003: whitefly; Nelson, 2007: aphid; Škaloudová et al., 2007: spider mite; Walzer & Schausberger, 2009: thrips). For instances, adult whiteflies have been shown to learn to avoid plants harboring predators of their offspring (Nomikou et al., 2003). In addition, adult thrips avoided investing their oviposition potential when predation risk is present compared to when the risk is absent (Walzer & Schausberger, 2009).

Non-consumptive predator effects can have a gradual and pervasive impact on resource use by prey. It is therefore crucial, especially in the context of biological control, to examine how strongly and widely predation risks induce the changes in prey foraging behaviors and whether these changes have cascading impacts on the magnitude and distribution of plant damage caused by herbivores. This question has been proposed as an important issue to resolve in quantifying the importance of non-consumptive predator effects (Lima, 1998), but there have been few attempts to address this question either in natural or managed ecosystems (but see Costamagna & Landis, 2011).

In pest management, understanding spatial and temporal scales of non-consumptive predator effects could be important to maximizing the effectiveness of biological control programs. In particular, induced changes in pest distributions on plants by non-consumptive predator effects should be explicitly considered to improve the augmentative release of natural enemies. In the study reported here, we examined habitat choice patterns by adult whiteflies (Hemiptera: Aleyrodidae) in response to predators of their offspring at different spatial scales. Predator-prey interactions in whiteflies provide a unique opportunity to study non-consumptive pathways because immature whiteflies are sessile and vulnerable to predation. In contrast, adult whiteflies are mobile and invulnerable to most predation. Thus, most attention has focused on the density-mediated interactions between the prey and predators via direct predation or parasitism on immature whiteflies. However, the life history of the prey suggests that evolutionary processes might favor female adults that can recognize predation risks and accordingly avoid risky habitats for their offspring (Ballabeni et al., 2001). Indeed, Nomikou et al. (2003) demonstrated that adult whiteflies (*Bemisia tabaci*) learn to avoid plants harboring predatory mites (*Amblyseius swirskii*). In addition, the presence of predatory beetles (*Delphaastus catalinae*) on poinsettia (*Euphorbia pulcherrima*) induced more whiteflies to move onto predator-free eggplant (*Solanum melongena*) (Lee et al., 2011).

The previous studies have shown that adult whiteflies avoid plants harboring predators of their offspring and this induced behavior influences host plant choice (Nomikou et al., 2003; Lee et al., 2011). In these studies, whitefly response was measured at a single spatial scale consisting of a small group of potted plants

However, little information is available as to whether adult whiteflies may respond to predators at smaller spatial scales such as within a plant canopy or how the application of predators may influence pest distributions at larger scales such as those in a greenhouse.

The objective of this study was to understand how adult whiteflies, *B. argentifolii*, alter their habitat use patterns on greenhouse cucumbers in response to predators, *D. catalinae*, at three spatial scales. We first measured the influence of *D. catalinae* on the settling of adult female whiteflies on a single cucumber leaf. We then asked whether adult whiteflies change their within-plant distribution in response to the predators. Finally, we determined whether the presence of *D. catalinae* on a cucumber plant with a high density of whiteflies can elevate pest dispersal from the heavily-infested plant into uninfested plants in a greenhouse.

Materials and Methods

Insects and plants

A *Bemsia argentifolii* (*B. tabaci* biotype B) colony was maintained on poinsettia (*Euphorbia pulcherrima*; cv 'Freedom Red' or 'Prestige Red'), in a walk-in growth chamber at 20-27^{o}C and 14:10 (L:D) photoperiod. The poinsettias in the insect colony were fertilized (N-P-K: 21-5-20) 5 days a week. Adult *Delphastus catalinae* were purchased from a commercial supplier (IPM Laboratories Inc., Locke, NY) and stored in a refrigerator <5 days at 10-15^{o}C. On the day of an experiment, *D. catalinae* were taken out of the refrigerator and released on white paper to collect vigorously moving adults. Cucumbers (*Cucumis sativus*; cv 'Marketmore 76') were grown in 15-cm pots

and fertilized 5 days a week in a greenhouse at 20-25^{o}C and 14:10 (L:D) photoperiod. The plant was tied to a bamboo stick to maintain its growth upright and side shoots were removed regularly. The plants were not treated with any pesticides.

Settling on leaf-disc

Leaf-discs (8 cm diameter) were excised from cucumbers (ca. 1 month old) and each leaf-disc was imbedded ventral side up in liquefied non-nutritious agar medium contained in a Petri dish lid (11 cm diameter). Solidified agar secured the leaf-disc and keeps the leaf tissue moisturized. The leaf-disc affixed in the agar medium was placed over a plastic container (10 cm diameter × 15 cm high) so that the underside of the leaf was within the container. Fifty adult *B. argentifolii* (mixed age and sex) were released into the container and allowed to oviposit for 36 hours, after which the whitefly adults were removed from the disc. The leaf-discs were examined under a microscope to verify that whitefly eggs had been oviposited. These eggs served as food for *D. catalinae* and stimulated feeding behaviors during experiments.

The leaf-discs with whitefly eggs were transferred and embedded onto new agar media in Petri dish lids as described above for use in the experiment. Each leaf-disc was then placed over the bottom half of the Petri dish (1.5 cm high), so that the underside of the leaf was facing down in the Petri dish. In the predator treatment, an adult *D. catalinae* was introduced into the Petri dish arena by placing the insect on the leaf-disc. The insect was allowed to acclimate for 0.5–1 hours. Controls received no predators. A female whitefly (ca. 2 days old) was aspirated into a glass pipette and gently blown into each Petri dish arena. Then, the experimental areas were randomly

placed on a transparent acetate floor (60 × 150 cm) secured on a wooden frame (60 cm high). This allowed observing insects in the Petri dish arena through the transparent acetate floor without disturbing the experimental arenas. After arranging the experimental arenas for observation, the insects were allowed to acclimate for one hour before data were recorded. The locations of the insects in Petri dish arenas (on leaf-disc vs. off leaf-disc) were recorded every 20 min for 6 hours by examining the inside of the arenas with the help of a mirror. In addition to their location, the activities of the two insects (walking vs. stationary) were also recorded. The experiment was conducted in a laboratory at 22-24^{o}C and with supplement florescent lights. Twenty-one experimental arenas were observed for each treatment.

To analyze settling patterns of adult whiteflies on the leaf-disc, the record was expressed as the time durations from the onset of observation until the whitefly was spotted on the leaf-disc. In most cases, the whitefly that had been spotted on a leaf-disc remained on the leaf-disc throughout the experiment (see Results). For this reason, survival analysis was used to compare times for whiteflies to settle on the leaf-disc between the two treatments. The time durations required for whiteflies to settle were modeled based on Klan-Meier survival curves and compared using a one-sided Wilcoxon-Gehan test in StatXact 8 (Cytel Inc., Cambridge, MA). In the predator treatment, the data were further analyzed to determine whether the location of *D. catalinae* (on the leaf-disc vs. off the leaf-disc) affected the settling of adult whiteflies on the leaf-disc. For this, the frequency of whiteflies on the leaf-discs when *D. catalinae* were on the leaf-disc was compared to that when *D. catalinae* were off the leaf-discs across all observations during the six hour test. In addition, the frequencies

of the *D. catalinae* activities (walking vs. stationary) were also compared between the two conditions (on leaf-disc vs. off leaf-disc). The frequencies were compared using a Pearson's chi-square test (JMP 8.0, SAS Institute, Cary, NC).

Within-plant distribution

Cucumbers (6 weeks old) were placed separately in BugDorm cages (45 × 45 × 90 cm; MegaView Science Education Services Co., Taichung, Taiwan) and two hundred adult whiteflies were released into each cage to infest the cucumber with immature whiteflies (eggs and nymphs). The immatures served as a food resource for *D. catalinae* during the experiment. After 5 days, all adult whiteflies were removed from each cucumber. The infested cucumbers (100 cm tall; with 10 leaves) were then placed in spun-bound polyester screen cages (ca. 150 × 150 × 150 cm) that were positioned on greenhouse benches. In each cage, three plants were placed 60 cm apart (center to center) in a triangular configuration. In the predator treatment cages, 20 adult *D. catalinae* were collected into a glass vial and released at the bottom of each plant, totaling 60 beetles per cage. In the control cages, no predators were released. After one day, 100 adult whiteflies were released at the bottom of each plant, totaling 300 whiteflies per cage. The numbers of adult whiteflies and *D. catalinae* were recorded on the underside of each leaf at 0.2, 1, 3, and 5 days after releasing the whiteflies. The experiment was conducted in a research greenhouse at 20-25^{o}C and 14:10 (L:D) photoperiod. The test was replicated 3 times.

For data analysis, the numbers of adult whiteflies observed on leaves were pooled into lower and upper plant stratums. Each plant stratum consisted of 5 or 6

leaves depending on observation time because the number of leaves increased from 10 to 12 due to plant growth during the study. The response variable was expressed as the proportion of adult whiteflies on the upper stratum relative to the total number of whiteflies on the plants at each observation. The data were analyzed using repeated measures ANOVA because the number of whiteflies was recorded repeatedly on the same plants in each experimental unit. In the ANOVA, the main factors were time, treatment, and their interactions. The linear model was analyzed using Proc MIXED in SAS (SAS Institute, Cary, NC). A covariance structure was selected based on model fit statistics.

Among-plant distribution

The objective of this experiment was to determine whether the application of *D. catalinae* on a cucumber with a high whitefly density (hereafter referred as a 'hotspot plant') induced elevated dispersal of adult whiteflies from the hotspot plant to other uninfested plants in a greenhouse. Before the test, cucumbers (5-6 weeks old) were infested with immature whiteflies (<10 days old) to create the hotspot plants. The immatures served as a food resource for *D. catalinae* during the experiment. The hotspot plants infested only with immature whiteflies were placed individually in BugDorm cages (45 × 45 × 90 cm), and 400 adult whiteflies were released into each cage. The adult whiteflies were allowed to settle on the cucumber for 24 hours and the number of whiteflies on each plant was recorded. The numbers of adult whiteflies on the hotspot plants were adjusted to an equal number (ca. 350) by aspirating extra whiteflies.

Experimental arenas were created in research greenhouse compartments at 20-25^{o}C and 14:10 (L:D) photoperiod. In each greenhouse compartment (4.0 × 6.5 m), 54 uninfested cucumbers (5-7 weeks old; ca 80 cm tall) were placed into a 5 × 11 (column × row) layout on greenhouse benches (3 × 6 m) with no plant at the center of the layout. A single hotspot plant (6-7 weeks old; ca. 90 cm tall) was placed at the center of each greenhouse compartment. In the predator treatment, 30 adult *D. catalinae* were released from a glass vial placed on the bottom of the hotspot plant; a sham glass vial with no predators was placed on the same location in the control. The numbers of adult whiteflies and *D. catalinae* were recorded on each plant at 0.1, 1, 3, 5, and 7 days. The experiment was replicated 4 times.

The data were first analyzed using repeated measures ANOVA for the number of adult whiteflies on the hotspot plant (one plant) and for the number of whiteflies on the surrounding plants (54 plants). In the ANOVA, the fixed factors were time, treatment, and their interactions; the random factor was the date of replication. The data were analyzed in SAS as described above. In addition to comparing the whitefly densities on plants, the spatial distribution of adult whiteflies among plants was analyzed using SADIE (Spatial Analysis by Distance IndicEs) (Perry, 1998). This was done to determine whether the presence of *D. catalinae* on the hotspot plant influenced the dispersal patterns of adult whiteflies from the hotspot plant to the uninfested plants compared to those under no predation risk. In SADIE, the distance to crowding, *C*, is defined as the minimum value of the total distance that individuals in the sample must move so that all are congregated in one location (see Perry, 1998 for details). For data that comprise a single cluster (e.g., whiteflies on the hotspot plants),

a smaller value of C indicates a more spatially-aggregated pattern around the single cluster. For this reason, the index of aggregation J_a, standardized from the distance C, was used as an estimate of the average dispersal distances of individual whiteflies from the hotspot plant. The index was calculated using the SADIEShell software (Rothamsted Experimental Station, Hertfordshire, UK).

Results

Settling on leaf-disc

Adult whiteflies delayed settling on the leaf-disc when *D. catalinae* was present, compared to whiteflies with no predators (Z = -1.81; P = 0.035) (Figure 5.1). When no predator was present, 90% of the adult whiteflies immediately settled on the leaf-disc and by 220 min all of the whiteflies had done so. When the predators were present, the proportion of adult whiteflies on the leaf-disc was 71% (67-76%) during the first 100 min and then the proportion increased for the following 60 min to the same level as observed in the absence of the predators.

In the predator treatment, the actual residence of *D. catalinae* on the leaf-disc significantly reduced the likelihood for whiteflies to be on the leaf-disc (χ^2 = 26.55, d.f. = 1, P < 0.0001). When *D. catalinae* were observed on the leaf-disc, 20% of the whiteflies did not settle on the leaf-disc; whereas when *D. catalinae* were not observed on the leaf-disc, 3% of the whiteflies did not settle on the leaf-disc (Figure 5.2A). In addition, there was a significant difference in the *D. catalinae* activities between when the beetle was present on the leaf-disc vs. off the leaf-disc. When *D. catalinae* were observed on the leaf-disc, 97% of the predators were stationary; whereas when *D.*

catalinae were observed off the leaf-disc, 67% of the predators were stationary (χ^2 = 58.14, d.f. = 1, P<0.0001) (Figure 5.2B).

Within-plant distribution

A majority of the adult whiteflies that were released from the bottom of the plant first settled on the lower leaves of the plant, regardless of the presence of *D. catalinae* on the plant (Figure 5.3). Five hours after releasing the whiteflies, 97 and 95% of adult whiteflies were found on the bottom 5 leaves in the control and predator treatments, respectively. Thereafter, adult whiteflies in the predator treatment moved upward significantly faster, compared to those in the control ($F_{3,12}$ = 5.96, P = 0.010). The proportion of whiteflies on the upper leaves of the plant stratum increased over 5 days to 32% in the control, whereas the proportion increased to 47% in the predator treatment (Figure 5.3). Most *D. catalinae* were observed on the lower leaves of the plant throughout the study and the mean number of *D. catalinae* on the plants decreased from 23.67 to 9.33 over 5 days (data not shown).

Among-plant distribution

Adult whiteflies continuously dispersed from the hotspot plant, where the whiteflies had been initially introduced, to uninfested cucumber plants in the greenhouse (Figure 5.4). There were fewer whiteflies on the hotspot plant where predators were released compared to the control based on the repeated measures of whitefly numbers throughout time across 5 observations ($F_{1,6}$ = 7.48, P = 0.0340). Along with the decrease in the whitefly numbers on the hotspot plant, there were increases in the

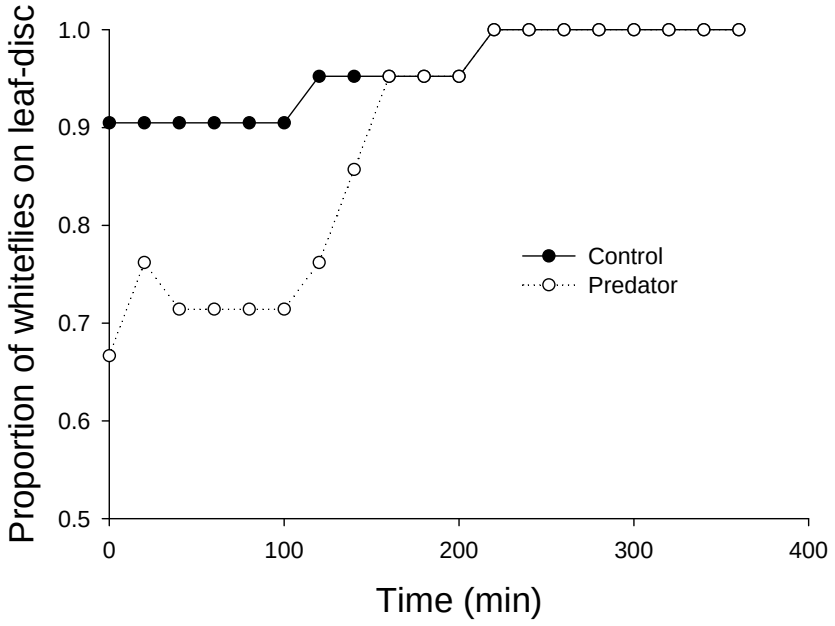

Figure 5.1. The proportion of adult whiteflies on the cucumber leaf-disc in the Petri-dish experimental arena over time. In the control, a single female whitefly was introduced into an experimental arena. In the predator treatment, a female whitefly and an adult *D. catalinae* were introduced.

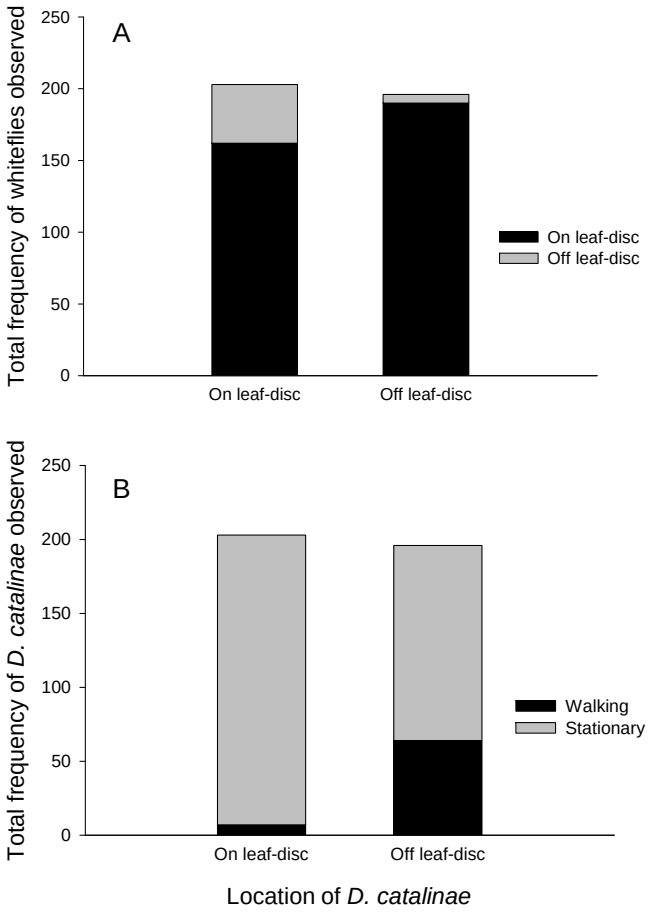

Figure 5.2. A) Total frequency of adult whiteflies observed on the leaf-disc vs. off the leaf-disc during the study in the two conditions: when *D. catalinae* were on the leaf-disc or when *D. catalinae* were off the leaf-disc. B) Total frequency of *D. catalinae* observed as walking or stationary during the study in the two conditions: when *D. catalinae* were on the leaf-disc or when *D. catalinae* were off the leaf-disc.

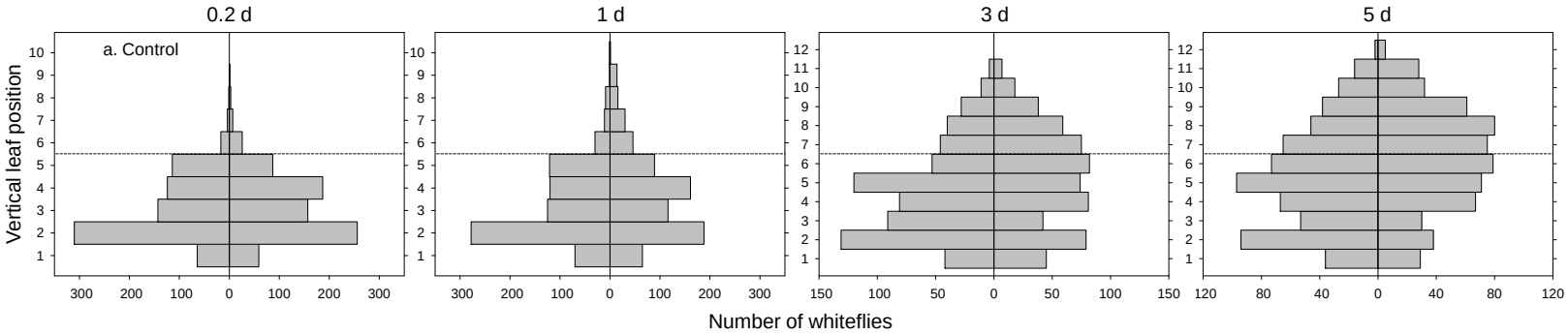

Figure 5.3. The vertical distributions of adult whiteflies on the leaves of cucumber plants over time. For the leaf position, a smaller number indicates an older and lower leaf.

whitefly numbers on the uninfested cucumber plants in both treatments; however, numbers of whiteflies on the uninfested cucumbers were not statistically different between the two treatments ($F_{1,6} = 0.24$, $P = 0.6399$).

For the spatial distributions of adult whiteflies, the index of aggregation J_a was not statistically different between the two treatments at any given observation time (t-test; $P>0.05$) (Figure 5.5). Here, a greater J_a indicates that individual adult whiteflies on average were located further from the hotspot plant in the greenhouse. Thus, the results support that there was no significant difference in the dispersal distances of individual whiteflies from the hotspot plants between the two treatments. Most *D. catalinae* were observed on the hotspot plant during the study, on which the mean number of *D. catalinae* decreased from 15.0 to 2.5 beetles per plant over time (Figure 5.6).

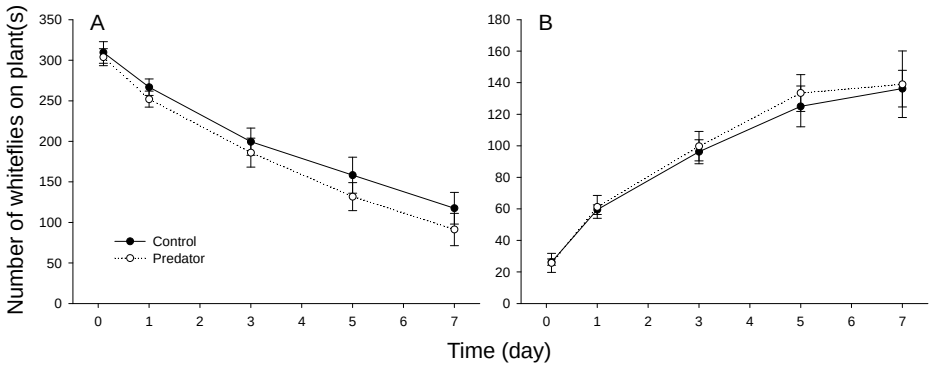

Figure 5.4. A) The mean number of adult whiteflies (± SEM) on the hotspot cucumber plant (n = 1) in the research greenhouse experiment over time. The hotspot plant was initially infested with adult whiteflies and placed in the middle of the greenhouse at the onset of the experiment. B) The mean number of adult whiteflies (± SEM) on the uninfested cucumber plants (n = 54) in the research greenhouse experiment over time.

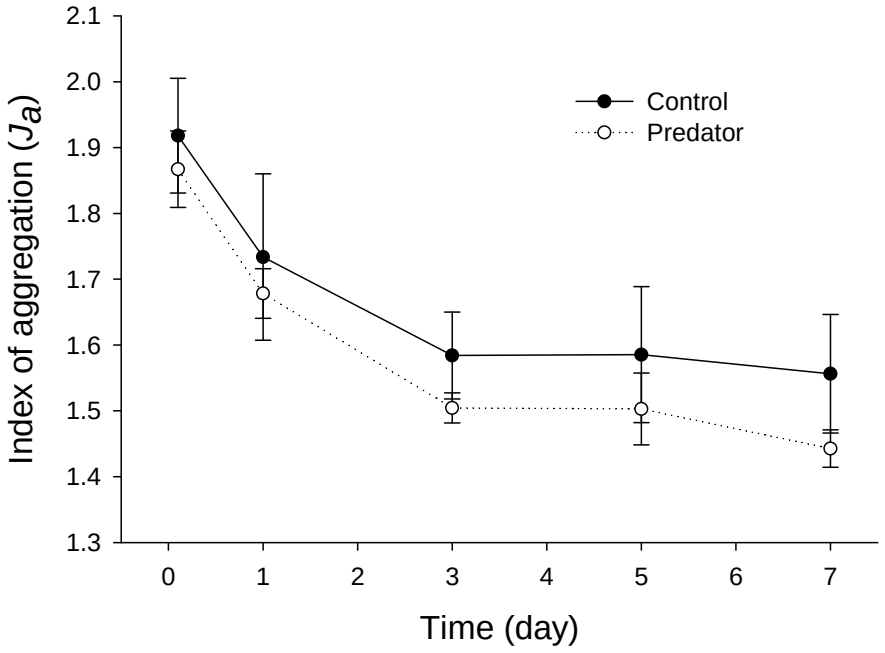

Figure 5.5. The mean index of aggregation of adult whiteflies (± SEM) in the research greenhouse experiment. The aggregation index was calculated using SADIE based on the distance to crowding. A greater value indicates adult whiteflies were aggregated around the hotspot plants in the greenhouse (see the text for detail).

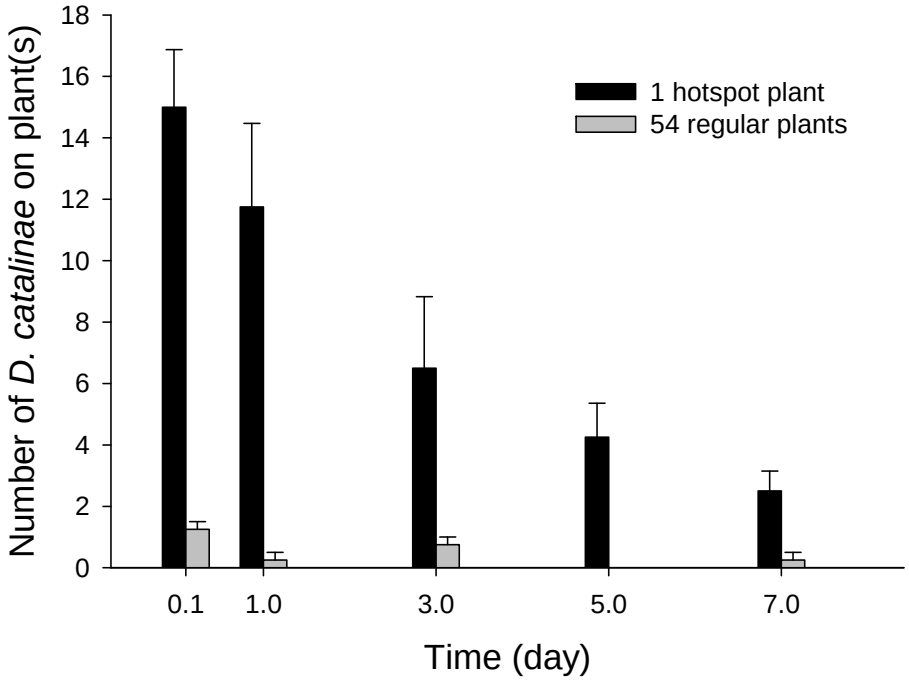

Figure 5.6. The mean number of adult *D. catalinae* (± SEM) on the hotspot plant and on the uninfested plants in the research greenhouse experiment over time. Thirty *D. catalinae* were released from the bottom of the hotspot plant.

Discussion

Considerable effort has been devoted toward development of biological control programs to manage whitefly pests (Gerling et al., 2001; Naranjo, 2001). Most of these studies have focused on the ability of natural enemies to consume sessile immature whiteflies. Among commercially-available biological control agents, a whitefly specialist predator, *D. catalinae*, has received attention for their ability to consume a large number of immature whiteflies (Hoelmer et al., 1993). In contrast, the potential impacts of this predatory beetle on adult whiteflies have not been well studied. This study measured the behavioral responses of adult whiteflies to predators at three spatial scales ranging from a small leaf-disc to a research greenhouse with more than 50 plants. Although there was variation across the experimental settings in the significance of predator avoidance behaviors by adult whiteflies, adult whiteflies in general responded to the predators by avoiding habitats in which the whiteflies were more likely to encounter predators.

In the small Petri-dishs arena provided with leaf-discs, most adult whiteflies readily settled on the leaf-disc when no predators were present, whereas whiteflies significantly delayed settling when predators were present. The actual residence of *D. catalinae* on the leaf-disc significantly reduced the likelihood for adult whiteflies to settle on the leaf-disc. It is notable that all the whiteflies eventually settled on the leaf-disc in 6 hours even when predators were present. This suggests that the adult whiteflies under predation risk are likely to settle on the leaf after a certain time threshold rather than consistently rejecting the plant, though the confinement to the leaf disc in the Petri dishes provided no habitat alternatives. Nonetheless, because

adult whiteflies that do not settle on plants are highly vulnerable to death within 24 hours due to starvation or dehydration (Fenigstein et al., 2001; Lee et al., 2010), adult whiteflies faced with no alternative, probably settle on plants even with high predation risks. In addition, it is also noteworthy that predators rarely moved around once they settled on the leaf-disc, presumably by devoting to feeding or resting. This reduced movement of the predators may lead the whiteflies to accept the leaf-disc.

The presence of *D. catalinae* also altered the within-plant distribution of adult whiteflies. In general, regardless of the presence of predators on the cucumber plants, adult whiteflies moved upward within a plant canopy after being released from the bottom of the plants. However, the presence of predators resulted in adult whiteflies moving upward faster compared to whiteflies in the control. In the predator treatment, most *D. catalinae* were present on the lower part of the plant in which their food resource (i.e., immature whiteflies) was relatively abundant. Therefore, it is likely that predation risk by *D. catalinae* was vertically different across plant height. It has been demonstrated that immature whiteflies are more abundant below the middle stratum of the plant (Hou et al., 2007) and *D. catalinae* are arrested by the presence of whitefly immatures (Guershon & Gerling, 2006). Therefore, the observed whitefly movement pattern supports the hypothesis that adult whiteflies moved preferentially to the upper plant strata to secure habitat for progeny with reduced predation risk.

The use of *D. catalinae* has been recommended for treating whitefly hotspots because the predators need a large number of whitefly eggs (between 100 and 150) to initiate and maintain their oviposition (Hoelmer et al., 1993), and also the beetles are costly ($0.15-0.29 / beetle; IPM Laboratories Inc., Locke, NY). For this reason, we

specifically examined whether applications of *D.catalinae* onto a whitefly hotspot resulted in elevated escape of adult whiteflies from the hotspot to uninfested plants in a research greenhouse. When adult *D. catalinae* were applied on a plant with a high whitefly density, adult whiteflies increased their dispersal from the whitefly hotspot plant to uninfested plants compared to when no predators were applied. However, the predator effect was quite modest. Although the presence of predators on the hotspot plant induced slightly but significantly more whiteflies to leave the hotspot plant, there was no significant difference in the accumulations of whiteflies on surrounding plants between the two treatments. This suggests that the presence of predators elevated the whitefly dispersal out from the hotspot plant but most of the extra dispersers did not re-settle on the plants. For this reason, the presence of predators did not cause an elevated whitefly infestation on the plants in the greenhouse. In addition, the presence of predators did not induce individual whiteflies to disperse further from the hotspot plant, compared to whiteflies on the hotspot plant with no predators.

On the hotspot plant, most *D. catalinae* were aggregated at the lower part of the plant throughout the study. Therefore, the predation risk was localized vertically within the cucumber plant. The same pattern was observed in the within-plant experiment as described above. In such circumstances, it is possible that upward within-plant movements of adult whiteflies may provide sufficient refuge at the upper stratum of the plant, thus ending their avoidance behavior. In addition, predator abundance on the hotspot plant decreased rapidly over time, and they were rarely found on other plants during the study. Therefore, it appears that a relatively higher predation risk was created temporarily on the hotspot plant during the early period of

the experiment but the risk might have waned rapidly thereafter. This may explain in part why the predator effect on whitefly dispersal was very modest. Further studies are warranted to examine whether predator density on the plant can influence the magnitude of avoidance behaviors of adult whiteflies, especially their dispersals among plants.

The results of this study indicate that the relative magnitude of the predator effects on the habitat use patterns by adult whiteflies appears to be higher at a within-plant scale compared to an among-plant scale. This might be viewed as having a far less striking impact on pest management compared to direct removal of prey by voracious predation of *D. catalinae* (Hoelmer et al., 1993). Nonetheless, it should not be underestimated that the altered habitat use pattern of adult whiteflies can substantially facilitate the use of unexplored habitat (e.g., the upper part of plants) by the pests. This distributional change of a whitefly population would be more important to consider when a cash crop has a low economic threshold such as greenhouse vegetables or ornamentals. Therefore, additional efforts are warranted to estimate a relative role of consumptive and non-consumptive effects of the predators, especially in the context of crop value and habitat structure on the crop.

REFERENCES

Costamagna AC & Landis DA (2011) Lack of strong refuges allows top-down control of soybean aphid by generalist natural enemies. Biological Control 57: 184–192.

Fenigstein A, Eliyahu M, Gan-Mor S & Veierov D (2001) Effects of five vegetable oils on the sweetpotato whitefly *Bemisia tabaci*. Phytoparasitica 29: 197–206.

Gerling D, Alomar O & Arnó J (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Protection 20: 779–799.

Guershon M & Gerling D (2006) Effects of plant and prey characteristics on the predatory behavior of *Delphastus catalinae*. Entomologia Experimentalis et Applicata 121: 15–21.

Heinz KM, Van Driesche RG & Parrella MP (2004) Biocontrol in protected culture. Ball Publishing, Batavia, IL, USA.

Hoelmer KA, Osborne LS & Yokomi RK (1993) Reproduction and feeding behavior of *Delphastus pusillus* (Coleoptera: Coccinellidae), a predator of *Bemisia tabaci* (Homoptera: Aleyrodidae). Journal of Economic Entomology 86: 322–329.

Hou M, Lu W & Wen J (2007) Within-plant distribution of *Bemisia tabaci* (Homoptera: Aleyrodidae) adults and immatures on greenhouse-grown winter cucumber plants. Journal of Economic Entomology 100: 1160–1165.

Lee D-H, Nyrop JP & Sanderson JP (2010) Effect of host experience of the greenhouse whitefly, *Trialeurodes vaporariorum*, on trap cropping effectiveness. Entomologia Experimentalis et Applicata 137: 193–203.

Lee D-H, Nyrop JP & Sanderson JP (2011) Avoidance of natural enemies by adult

whiteflies, *Bemisia argentifolii*, and effects on host plant choice. Biological Control 58: 302–309.

Lima SL (1998) Nonlethal effects in the ecology of predator-prey interactions. BioScience 48: 25–34.

Luttbeg B & Kerby JL (2005) Are scared prey as good as dead? Trends in Ecology and Evolution 20: 416–418.

Naranjo SE (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop Protection 20: 835–852.

Nelson EH (2007) Predator avoidance behavior in the pea aphid: costs, frequency, and population consequences. Oecologia 151: 22–32.

Nelson EH, Matthews CE & Rosenheim JA (2004) Predators reduce prey population growth by inducing changes in prey behavior. Ecology 85: 1853–1858.

Nelson EH & Rosenheim JA (2006) Encounters between aphids and their predators: the relative frequencies of disturbance and consumption. Entomologia Experimentalis et Applicata 118: 211–219.

Nomikou M, Janssen A & Sabelis MW (2003) Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. Oecologia 136: 484–488.

Perry JN (1998) Measures of spatial pattern of counts. Ecology 79: 1008–1017.

Preisser EL, Bolnick DI & Benard MF (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86: 501–509.

Preisser EL, Bolnick DI & Grabowski JH (2009) Resource dynamics influence the strength of nonconsumptive predator effects on prey. Ecology Letters 12: 315–

323.

Relyea RA (2001) The relationship between predation risk and antipredator responses in larval anurans. Ecology 82: 541–554.

Škaloudová B, Zemek R & Křivan V (2007) The effect of predation risk on an acarine system. Animal Behaviour 74: 813–821.

Thaler JS & Griffin CAM (2008) Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. Entomologia Experimentalis et Applicata 128: 34–40.

Walzer A & Schausberger P (2009) Non-consumptive effects of predatory mites on thrips and its host plant. Oikos 118: 934–940.

Werner EE & Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.

CHAPTER 6

SUMMARY AND RESEARCH IMPLICATIONS

Traditionally, whitefly management has largely relied upon applications of insecticides which, in turn, have usually resulted in whiteflies developing resistance to these compounds (Oliveira et al., 2001). Resistance to insecticides and other negative impacts of pesticide use on human health and the environment have prompted efforts to enhance cultural and biological control of whiteflies (Faria & Wraight, 2001; Gerling et al., 2001; Hilje et al., 2001; Naranjo, 2001).

Trap cropping is a biologically based alternative or supplement to synthetic pesticides in which a preferred host plant is used to attract target pests away from a cash crop (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). This non-toxic management tactic can offer significant economic and environmental benefits and can be integrated with other management tactics. However, the results of this study indicate that trap cropping has only a limited potential for either *Bemisia argentifolii* or *Trialeurodes vaporariorum* on greenhouse poinsettia (Chapter 2). More importantly, experiments revealed that the potential of trap cropping must be interpreted differently for *B. argentifolii* and *T. vaporariorum*, because there was a clear difference in the response to an eggplant trap crop by the two whitefly species.

Eggplant was not highly attractive to adult *B. argentifolii*, and therefore failed to draw a sufficient number of adult whiteflies away from a poinsettia cash crop to function as a trap crop. In contrast, eggplant was highly attractive to adult *T.*

vaporariorum; however, high mortality of adult *T. vaporariorum* on a poinsettia cash crop diluted the effectiveness of trap cropping even when a large number of adults were attracted to the trap crop. The number of the adult whiteflies decreased on poinsettias in monoculture as fast as the number of the adults decreased on poinsettias under the trap cropping. That is, most of adult *T. vaporariorum* found on the trap crop would have died on the cash crop if eggplant was not provided as a trap crop.

These results show that pest mortality on a cash crop, in conjunction with pest attraction to a trap crop, can significantly influence the effectiveness of trap cropping. Thus, for *T. vaporariorum*, the observed strong attraction to eggplant might be useful as a trap crop system when whitefly mortality on a poinsettia cash crop is not high. Because *T. vaporariorum* is a generalist, the performance and preference of this insect on a given plant is strongly affected by prior host plant experience (van Lenteren & Noldus, 1990). Under the trap crop system, experience of whiteflies on a poinsettia cash crop is predicted to enhance their survivorship and concurrently decrease their preference for an eggplant trap crop. This trade-off would influence the effectiveness of trap cropping. Experiments showed that experience of *T. vaporariorum* on a poinsettia cash crop enhanced their survivorship on poinsettias while maintaining their strong preference to an eggplant trap crop (Chapter 3). As expected, this change contributed to an increase in the effectiveness of the trap cropping. Still, as adult whitefly survivorship on the cash crop was relatively low even after the pests were reared on the cash crop for several generations, it is noteworthy that a large proportion of adult whiteflies on the trap crop did not result in a reciprocal density reduction in the cash crop. This emphasizes that pest mortality on a cash crop strongly influences

trap cropping effectiveness and the accumulation of adult whiteflies on a trap crop should not be interpreted as a direct measure of trap cropping effectiveness.

In the previous study (Chapter 2), eggplant failed to draw a sufficiently large number of *B. argentifolii* from a poinsettia cash crop to function as a trap crop. Cucumber was further tested as a trap crop for *B. argentifolii* in the same context; however, a similar pattern was observed in the experiment (Chapter 4). When adult *B. argentifolii* were provided an initial choice between poinsettia and cucumber, the adults strongly preferred cucumber over poinsettia. However, cucumber did not effectively draw adult whiteflies from a poinsettia cash crop if the adults had first settled on the poinsettia. That is, adult *B. argentifolii* readily accept a poinsettia cash crop upon landing and do not further respond to a more-preferred trap crop. This altered response of the whiteflies to the trap crop, after having settled on the cash crop, is a challenge to successful trap cropping for *B. argentifolii*.

Under such circumstances, limitations to the trap cropping might be mitigated by integrating a "push component" into the system to induce more whiteflies to leave the cash crop and subsequently move to the trap crop. To test this, manipulative experiments were conducted to determine whether adult whiteflies avoid natural enemies of their offspring and whether enemy-avoidance behaviors by whiteflies can be used to induce the whiteflies to leave the cash crop and move onto an enemy-free trap crop (Chapter 4). In the study, the presence of predatory beetles, *Delphastus catalinae*, on a poinsettia cash crop induced significantly more whiteflies to disperse to a cucumber trap crop compared to poinsettia with no natural enemies. However, it is crucial to further evaluate how the push effect plays a role in field settings and for a

longer period. For instance, it is possible that dispersal of *D. catalinae* onto a trap crop can, in turn, dilute the intrinsic attractiveness to the trap crop to whiteflies.

Further experiments were conducted to better understand how adult *B. argentifolii* alter their habitat use patterns in response to the predators across different spatial scales (Chapter 5). Little information is available how prey modulate their predator-avoidance behaviors across different habitat scales, especially in the context of biological control (but see Costamagna & Landis, 2011). This knowledge would be crucial to evaluate whether predator-avoidance behaviors by whiteflies occur at the level of importance to pest management. Results show that adult whiteflies delay settlings on plant leaves when predators are present. In addition, whiteflies utilized upper plant strata as predator-free space at a within-plant scale to avoid predators harboring lower plant parts. Although the presence of predators induced more whiteflies to disperse out from a plant harboring predators compared to the plant with no predators, this did not result in a reciprocal extra increase in the whitefly numbers on surrounding plants compared to when predators were not applied. Therefore, the observed predator-avoidance behaviors may have negative effects on management efficacy at the within-plant scale by elevating the pest dispersal into a new habitat area; however, the predator effects were quite marginal at the among-plant scale.

The current study explored how adult whiteflies change their habitat use patterns under trap cropping (i.e., bottom-up factors) and in response to natural enemies of their offspring (i.e., top-down factors). The study also examined how behavioral changes by whiteflies can influence the pest management efficacy. The following summarize the main findings and their implications for pest management:

1. Two co-occurring whitefly species, *T. vaporariorum* and *B. argentifolii*, on a poinsettia cash crop responded to a common eggplant trap crop in very different manners and thereby their implications for pest management must be interpreted differently (Chapter 2).

2. Adult *B. argentifolii* readily accepted a poinsettia cash crop upon landing and did not further respond to a more-preferred trap crop. Therefore, the trap cropping failed to function as a management tool (Chapters 2 & 4).

3. Adult *T. vaporariorum* strongly preferred and moved to an eggplant trap crop, regardless of their host experience on a poinsettia cash crop. However, high mortality of adult *T. vaporariorum* on the cash crop significantly diluted the effectiveness of trap cropping even when a large number of adults were attracted to the trap crop. For this reason, there was only a marginal management benefit from the trap cropping (Chapters 2 & 3).

4. The effectiveness of trap cropping is governed by the interplays between pest attraction to a trap crop and pest mortality on a cash crop. Therefore, accumulation of pests on a trap crop should not be directly interpreted as a measure of trap cropping effectiveness (Chapters 2 & 3).

5. The presence of predators, *D. catalinae*, on a poinsettia cash crop induced more adult *B. argentifolii* to leave the cash crop and move onto a cucumber trap crop, compared to whiteflies on the cash crop with no predators. This supports that the efficacy of trap cropping can be improved by the push effect from *D. catalinae* (Chapter 4).

6. Adult *B. argentifolii* modulated their avoidance behaviors to *D. catalinae* across different spatial scales. The predator-avoidance behavior may have a negative implication at within-plant scale by inducing more whiteflies to move into upper plant strata. However, the effect was marginal at among-plant scale (Chapter 5).

7. Predator-avoidance behaviors by whiteflies can have both positive and negative impacts on pest management efficacy, depending on the management systems and the spatial scales of interests. Therefore, additional effort should be made to assess and better incorporate these non-consumptive predator effects into the pest management programs (Chapters 4 & 5).

REFERENCES

Costamagna AC & Landis DA (2011) Lack of strong refuges allows top-down control of soybean aphid by generalist natural enemies. Biological Control 57: 184–192.

Faria M & Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. Crop Protection 20: 767–778.

Gerling D, Alomar O & Arnó J (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Protection 20: 779–799.

Hilje L, Costa HS & Stansly PA (2001) Cultural practices for managing *Bemisia tabaci* and associated viral diseases. Crop Protection 20: 801–812.

Hokkanen HM (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119–138.

Naranjo SE (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop Protection 20: 835–852.

Oliveira MRV, Henneberry TJ & Anderson P (2001) History, current status, and collaborative research projects for *Bemisia tabaci*. Crop Protection 20: 709–723.

Shelton AM & Badenes-Perez FR (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Van Lenteren JC & Noldus LPJJ (1990) Whitefly-plant relationships: behavioral and ecological aspects of whitefly-plant relationships. Whiteflies: Bionomics, Pest Status and Management (ed. by D Gerling), pp. 47–89. Intercept, Andover, UK.